A statistical mechanics approach to reverse engineering: sparsity and biological priors on gene regulatory networks

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The important task of determining the connectivity of gene networks, and at a more detailed level even the kind of interaction existing between genes, can nowadays be tackled by microarray-like technologies. Yet, there is still a large amount of unknowns with respect to the amount of data provided by a single microarray experiment, and therefore reliable gene network retrieval procedures must integrate all of the available biological knowledge, even if coming from different sources and of different nature. In this paper we present a reverse engineering algorithm able to reveal the underlying gene network by using time-series dataset on gene expressions considering the system response to different perturbations. The approach is able to determine the sparsity of the gene network, and to take into account possible a priori biological knowledge on it. The validity of the reverse engineering approach is highlighted through the deduction of the topology of several simulated gene networks, where we also discuss how the performance of the algorithm improves enlarging the amount of data or if any a priori knowledge is considered. We also apply the algorithm to experimental data on a nine gene network in Escherichia coli.

I. INTRODUCTION

The amount and the timing of appearance of the transcriptional product of a gene is mostly determined by regulatory proteins through biochemical reactions that enhance or block polymerase binding at the promoter region (Jacob and Monod 1961, Dickson et al. 1975). Considering that many genes code for regulatory proteins that can activate or repress other genes, the emerging picture is conveniently summarized as complex network where the genes are the nodes, and a link between two genes is present if they interact. The identification of these networks is becoming one of the most relevant task of new large-scale genomic technologies such as DNA microarrays, since gene networks can provide a detailed understanding of the cell regulatory system, can help unveiling the function of previously unknown genes and developing pharmaceutical compounds.

Different approaches have been proposed to describe gene networks (see (Filkov 2005) for a review), and different procedures have been proposed (Tong et al. 2002, Lee et al. 2002, Ideker et al. 2001, Davidson et al. 2002, Arkin et al. 1997, Yeung et al. 2002) to determine the network from experimental data. This is a computationally daunting task, which we address in the present work. Here we describe the network via deterministic evolution equations (Tegner et al. 2003, Bansal et al. 2006), which encode both the strength and the direction of interaction between two genes, and we discuss a novel reverse engineering procedure to extract the network from experimental data. This procedure, though remaining a quantitative one, realizes one of the most important goal of modern system biology, which is the integration of data of different type and of knowledge obtained by different means.

We assume that the rate of synthesis of a transcript is determined by the concentrations of every transcript in a cell and by external perturbations. The level of gene transcripts is therefore seen to form a dynamical system which in the most simple scenario is described by the following set of ordinary differential equations (de Jong et al. 2002):

\[ \dot{X}(t) = AX(t) + BU(t) \]  

where \( X(t) = (x_1(t), \ldots, x_{N_g}(t)) \) is a vector encoding the expression level of \( N_g \) genes at times \( t \), and \( U \) a vector encoding the strength of \( N_p \) external perturbations (for instance, every element \( u_k \) could measure the density of a specific substance administered to the system). In this scenario the gene regulatory network is the matrix \( A \) (of
time variation of the characteristics modes obtained via SVD. The matrix $B$ (of dimension $N_g \times N_g$) encodes the coupling of the gene network with the $N_p$ external perturbations, as $B_{ik}$ measures the influence of the $k$-th perturbation on the $i$-th gene.

A critical step in our construction is the choice of a linear differential system. Even if a such kind of model is based on particular assumptions on the complex dynamics of a gene network, it seems the only practical approach due to the lack of knowledge of the real interaction mechanism between thousands of genes. Even a simple nonlinear approach would give rise to an intractable amount of free parameters. However, it must also be recognized that all other approaches or models have weakness points. For instance, boolean models (which have been very recently applied to inference of networks from time series data, as in [Martin et al. 2007]), strongly discretize the data and select, via the use of an arbitrary threshold, among active and inactive gene at every time-step. Dynamical Bayesian models, instead, are more data demanding than linear models due to their probabilistic nature. Moreover, their space complexity grows like $N_g^4$ (at least in the famous Reveal Algorithm by K.P. Murphy [Murphy 2001]), which makes this tool suitable for small networks.

The linear model of Eq. (1) is suitable to describe the response of a system to small external perturbations. It can be recovered by expanding to first order, and around the equilibrium condition $X(t) = 0$, the dependency of $\dot{X}$ on $X$ and $U$, $\dot{X}(t) = f(X(t), U)$. Stability considerations ($X(t)$ must not diverge in time) require the eigenvalues of $A$ to have a negative real part. Moreover it clarifies that if the perturbation $U$ is kept constant the model is not suitable to describe periodic systems, like cell cycles for example, since in this case $X(t)$ asymptotically approaches a constant.

Unfortunately data from a given cell type involve thousands of responsive genes $N_g$. This means that there are many different regulatory networks activated at the same time by the perturbations, and the number of measurements (microarray hybridizations) in typical experiments is much smaller than $N_g$. Consequently, inference methods can be successful, but only if restricted to a subset of the genes (i.e. a specific network) [Basso et al. 2005], or to the dynamics of genes subsets. These subsets could be either gene clusters, created by grouping genes sharing similar time behavior, or the modes obtained by using singular value decomposition (SVD). In these cases it is still possible to use Eq. (1), but $X(t)$ must be interpreted as a vector encoding the time variation of the clusters centroids, or the time variation of the characteristics modes obtained via SVD.

In this paper we present a method for the determination of the matrices $A$ and $B$ starting from time series experiments using a Global Optimization approach to minimize an appropriate figure of merit. With respects to previous attempts, our algorithm as the uses explicitly the insight provided by earlier studies on gene regulatory networks [Jenog et al. 2000; Jenog et al. 2001], namely, that gene networks in most biological systems are sparse. In order to code such type of features the problem itself must be formulated as mixed-integer nonlinear optimization one [Hansen et al. 1993]. Moreover our approach is intended to explicitly incorporate prior biological knowledge as, for instance, it is possible to impose that: $A_{ij} < 0 \ (= 0, > 0, \neq 0)$ if it is known that gene $j$ inhibits (does not influence, activates, influences) gene $i$. This means that the optimization problem is subject to inequality and/or equality constraints. Summing up the characteristics of the problem we must solve: high dimensionality, mixed integer, nonlinear programming problem for the exact solution of which no method exists. An approximate solution can be found efficiently using a global optimization techniques [Horst and Pardalos 1992; Pardalos and Romeijn 2002] based on an intelligent stochastic search of the admissible set. As consequence of the optimization method used, there is no difficulties to integrates different time series data investigating the response of the same set of genes to different perturbations, even if different time series are sampled at different (and not equally spaced) time points. The integration of different time series is a major achievement, as it allows for the joint use of data obtained by different research groups. We believe that the integration of multiple time-series dataset in unveiling a gene network is a topic of great interest as focused in recently published papers [Shi et al. 2007].

We illustrate and test the validity of our algorithm on computer simulated gene expression data, and we apply it to an experimental gene expression data set obtained by perturbing the SOS system in bacteria E. coli.

II. METHODS

The simplest assumption regarding the dynamical response of gene transcripts (intially in a steady state, $X(t) = 0$ for $t < 0$), to the appearance of an external perturbation $U(t)$ at time $t > 0$ is given by Eq. (1). Since the state of the system measured at discrete times $t = t_k$, $k = 0, \ldots, N_t$, it useful to consider the discrete form of Eq. (1)

$$X(t_{k+1}) = AX(t_k) + \bar{U}(t_k, t_{k+1}),$$

where $A$ is a matrix with dimension $N_g \times N_g$, and $\bar{U}$ is a function of the perturbations, namely
\[ A = \exp(A\Delta t), \]
\[ \tilde{U}(t_k, t_{k+1}) = \int_{t_k}^{t_{k+1}} \exp(A(t_{k+1} - \tau)) B U(\tau) \, d\tau. \]

Here we have assumed, for simplicity sake, \( t_k = k\Delta \), but the generalization to the most general case is straightforward. In particular, for constant \( t \) we use as a cost function the reduced form:

\[ \chi^2_{\text{red}} = \frac{CF(A, B)}{n_{\text{dof}}\sigma^2}, \]

where

\[ CF(A, B) = \sum_{k=0}^{N_t-1} \left[ X(t_{k+1}) - (AX(t_k) + BU(t_k)) \right]^2 + \left[ X(t_k) - (A^{-1}X(t_{k+1}) + \tilde{U}(t_{k+1}, t_k)) \right]^2. \]

Note that \( A^{-1}\tilde{U}(t_k, t_{k+1}) = -\tilde{U}(t_{k+1}, t_k) \), and the quantities \( A \) and \( \tilde{U}(t_k, t_{k+1}) \) can be obtained from \( A \) and \( B \) by appropriate numerical approximation algorithms for Eq.s (3). The quantity \( \sigma \) denotes the standard deviation of the independent, additive noise affecting the dataset.

A straightforward optimization on dynamics/input matrices of Eq. (1) is the main improvement of the proposed approach with respect to the previous ones. This is the only way that enable us to incorporate the sparseness requirement on \( A, B \) and eventually available biological priors. It is clear that sparseness was destroyed by exponentiation and integration involved in the continuous-discrete transformation of the problem, in the same way simple bounds on \( A, B \) elements are transformed in highly complex nonlinear relations on \( A, B \). The price paid for the flexibility of the approach is the computational effort required for any computation of the error function. This put a very strong attention to the efficiency of the optimization algorithm.

In Eq. (6) the two contributions in square brackets account for the forward and backward propagation, respectively, and thus implement the time reversibility of the dynamics. Moreover, the sparsity of the gene network is taken into account via the number of degrees of freedom (d.o.f.) defined as \( n_{\text{dof}} = n_{\text{par}} - n_{\text{eq}} \) with \( n_{\text{par}} = N_g(N_g + N_p) - n_{\text{zero}} \).
the number of free parameters, \( n_{\text{eq}} = N_g(N_t - 1) \) the number of equations (constraints) and \( n_{\text{zero}} \) the number of elements of \( \mathbf{A} \) and \( \mathbf{B} \) (a total of \( n_{\text{zero}} \)) fixed to zero.

The generalization of the algorithm to the case in which there are different time-series, \( X^\alpha(t_k) \), corresponding to the response of the same set of genes to similar and/or different perturbations \( \mathbf{B}^\alpha \) with \( \alpha = 1, \ldots, N_p \) is straightforward. In this case the cost function to be minimized is simply

\[
\chi^2_{\text{red}} = \frac{1}{2n_{\text{dof}}} \sum_{\alpha=1}^{N_p} \frac{\mathcal{CF}(\mathbf{A}, \mathbf{B}^\alpha)}{\sigma_\alpha^2}.
\]  

(7)

Here we have assumed the noise to depend on the time-series (\( \alpha \)). It is clearly possible, however, to introduce a time (\( t_k \)) and even a gene (\( i \)) dependence, i.e. to use \( \sigma = \sigma_{ij}(t_k) \).

We detail now our procedure to find the spare matrices \( \mathbf{A} \) and \( \mathbf{B} \) minimizing \( \chi^2_{\text{red}} \), which is in general a formidable task. The first difficulty is the determination of the number \( n_{\text{par}} \) of not vanishing elements of \( \mathbf{A}, \mathbf{B} \) (or equivalently the number of d.o.f. \( n_{\text{dof}} \)). Having determined \( n_{\text{par}} \) the problem is still very complicated since there are

\[
\frac{(N_g(N_g + N_p))!}{n_{\text{par}}!(N_g(N_g + N_p) - n_{\text{par}})!}
\]

(8)

different ways of choosing these \( n_{\text{par}} \) elements out of the \( N_g(N_g + N_p) \) candidates. For typical values of the parameters, for instance \( N_g = 10 \) and \( n_{\text{par}} = 1/2N_g^2 = 50 \), the number of possible combinations is of the order of \( 10^{12} \), so big that any kind of extensive algorithmic procedure is precluded. A practical approach to, at least approximately solve, this formidable problem is that of resort to a global optimization techniques based on a stochastic strategy to search of the admissible set, for a comprehensive review of such type of methods one can see [Horst and Pardalos 1995, Horst and Pardalos 1995]. We have tackled this problem via the implementation of the more classical of such methods: a simulated annealing procedure [Kirkpatrick et al. 1983], based on a Monte Carlo dynamics. For each possible value of the number of parameters \( n_{\text{par}} \), the algorithm search for the matrices \( \mathbf{A} \) and \( \mathbf{B} \) with a total of \( n_{\text{par}} \) non zero elements minimizing the cost function of Eq. (8), as discussed below. We then easily determine \( \chi^2_{\text{red}}(n_{\text{par}}) \) and the minimizing matrices \( \mathbf{A}^* \) and \( \mathbf{B}^* \) which are our best estimates of the true matrices. In order to determine the matrices \( \mathbf{A} \) and \( \mathbf{B} \) with a total of \( n_{\text{par}} \) non zero parameters which minimize the cost function, our simulated annealing procedure starts with two random matrices \( \mathbf{A} \) and \( \mathbf{B} \) with a total of \( n_{\text{par}} \) not vanishing parameters, and changes the elements of these matrices according to two possible Monte Carlo moves. One move is the variation of the value of a not vanishing element of the two matrices, the other one consists in setting to zero a previously non-zero element, and to a random value a zero element. Each move, which involves a variation \( \Delta \mathcal{CF} \) of the cost function, is accepted with a probability \( \exp[-\Delta \mathcal{CF}/T] \), where \( T \) is an external parameter. As in standard optimization by annealing procedures, we start from a high value of \( T \), of the order of the cost function value, and then we slowly consider the limit \( T \to 0 \). In the limit of infinitesimally small decrease of \( T \) the algorithm is able to retrieve the true minimum of the cost function, while for faster cooling rates estimates of the real minimum are recovered.

As the Monte Carlo moves attempt to change the values of the elements of \( \mathbf{A} \) and \( \mathbf{B} \), it is easy to introduce biological constraints on the values of \( A_{ij} \) and of \( B_k \), as we will shown in a following example. The algorithm requires the evaluation of the cost function \( \mathcal{CF} \), which is a time consuming operation as the computation of the discrete matrix \( A \) and of its inverse \( A^{-1} \) are required. We have implemented this algorithm in C++ making use of the GNU Scientific Library, www.gsl.org.

III. RESULTS

In this section, we illustrate our reverse engineering algorithm with three examples. The validity of our algorithm and of other known ones are evaluated by comparing the exact dynamical matrices \( \mathbf{A} \) and \( \mathbf{B} \) with their best estimate \( \mathbf{A}^* \) and \( \mathbf{B}^* \) obtained via the reverse engineering procedure. To this end, we have introduced the parameter

\[
\eta_C = \frac{|C - C^*|}{|C|},
\]

(9)

where \( C^* = \mathbf{A}^* \) or \( \mathbf{B}^* \) and \( |C| \) is the \( L_2 \) norm of the matrix \( C \). Clearly, \( \eta_C \geq 0 \), the equality being satisfied if and only if \( C = C^* \). Since \( \eta_C \) is a measure of a relative error it has no upper bound, but the estimate of \( C \) becomes unreliable when \( \eta_C \) is above 1, i.e. \( |C - C^*| > |C| \). This parameter allows for a faithful evaluation of the quality of the reverse engineering approach, as it summarizes the comparisons of all retrieved elements \( C_{ij} \) with their true values \( C_{ij}^* \).

We discuss three applications. First, we show how our algorithm works when applied to a single time series. In this case one can show that the cost function \( \chi^2_{\text{red}}(\mathbf{A}, \mathbf{B}) \), which takes into account both the forward and the backward
FIG. 1: Synthetic time-series $X(t_k)$ with $N_g = 8$ elements measured at $N_t = 20$ equally spaced time-points.

propagation, is more effective in determining the structure of the gene network than the usual cost function $CF(A, B)$ of Eq. 4, which only considers the forward propagation. The second example shows how we can easily take into account the presence of different time-series, while the last example shows how biological priors can be included. Before discussing the examples we shortly describe the procedure used to generate the synthetic dataset.

1. Generation of a synthetic dataset

In order to generate a synthetic dataset $X(t_k)$ one must construct the matrices $A$ and $B$, from which it is possible to generate the noiseless time-series $\overline{X}(t_k)$. Hence, one gets $X(k) = \overline{X}(t_k) + \varepsilon_k$ for $k = 1, \ldots, N_t$ where $\varepsilon_k$ are i.i.d. random variables with standard deviation $\sigma$.

While there are no constraints on $B$, $A$ must be a sparse random matrix whose complex eigenvalues have negative real part. The generation of $A$ proceeds according the following steps. First, we generate a $N_g \times N_g$ block diagonal matrix $A^{(0)}$, whose $N_g$ blocks are $2 \times 2$ antisymmetric matrices with diagonal elements $\lambda^a$ and off diagonal elements $\lambda^i$, or $1 \times 1$ negative real elements $\lambda$. By direct constructions all of the $N_g$ eigenvalues of the matrix $A^{(0)}$ have negative real part. Then we generate a series $R_k$ of random unitary matrices, with only 4 off-diagonal not vanishing entries, and compute the matrices $A^k = R_k A^{(k-1)} R_k^{-1}$, all of them sharing the spectrum of $A^{(0)}$. Clearly, as $k$ grows, the number of vanishing entries (the sparsity) of $A^{(k)}$ decreases. We fix $A$ as the matrix $A^{(k)}$ characterized by the desired number of vanishing elements. By choosing typical values of $\lambda^a$ and $\lambda^i$ it is possible to control the time scale of the relaxation process of the system following the application of the perturbation.

2. Example 1: a single time series

Let us consider a simulated time-series $X(t_k) = (x_1(t_k), \ldots, x_{N_g}(t_k))$ with $N_g = 8$ measured at $N_t = 20$ equally-spaced time-points, as shown in Fig. 1. This dataset is generated by starting from a sparse gene network $A$ (with only 49 out of $N_g^2 = 64$ non-zero elements), a constant perturbation $U(t) = 1$ and a sparse external perturbation-coupling matrix $B$ with a single not vanishing entry. The white noise is characterized by a standard deviation

$$\sigma(p) = p \sum_{i=1}^{N_g} \sum_{k=1}^{N_t} \frac{|x_i(t_k)|}{N_g N_t},$$

measured in units of the mean absolute value of the expression levels of all genes. In particular the value $p = 0.05$ has been used.

We have applied our algorithm to this dataset. To this end, we have minimized the reduced chi-square $\chi_{\text{red}}^2$, defined in Eq. 5, for different values of the number of parameters $n_{\text{par}}$ (i.e. of the number of degrees of freedom $n_{\text{dof}}$). Fig. 2 shows that $\chi_{\text{red}}^2$ has a non-monotonic dependence on the number of parameters $n_{\text{par}}$. This feature is a signature of the fact that both networks with few or with many connections are bad descriptions of the actual gene regulatory system.
FIG. 2: The main panel (inset) show the dependence of $\chi^2_{\text{red}}$ (of the minimum of the cost function) on the number of not vanishing parameters $n_{\text{par}}$, as determined by our algorithm when applied to the time-series shown in Fig. The fluctuations are due to the probabilistic nature of the Monte Carlo minimization procedure. The quantity $\chi^2_{\text{red}}$ varies non-monotonically with $n_{\text{par}}$, and has a minimum with $n_{\text{par}} = 39$ parameters.

FIG. 3: We plot here the values of the element of the estimated matrices $A_{ij}^*$, obtained both with the linear algebraic approach and with our algorithm, versus their true value $A_{ij}$. Ideally, the points should line on the $y = x$ dotted line.

Accordingly, our best estimate of the number of not vanishing parameters is $n_{\text{par}}^* = 39$, where $\chi^2_{\text{red}}$ has its minimum, and the corresponding minimizing matrices $A^*$ (with 33 non zero entries) and $B^*$ (with 6 non zero elements) are our best estimates of the actual gene network encoding matrix $A$ and of the matrix $B$. The estimators assume the values $\eta_A = 0.76$ and $\eta_B = 0.005$. These values indicate that, when applied to this small dataset, our algorithm is able to retrieve $B$ to a very good approximation, and $A$ with a comparatively larger error.

For comparison, we have also obtained the matrices $A$ and $B$ which exactly minimize $CF(A, B)$ via a linear algebraic approach, and retrieved the corresponding continuous matrices via the use of the bilinear transformation, obtaining the scores $\eta_A = 2.1$ and $\eta_B = 0.012$. These numbers prove that by exploiting the time reversibility of the equation of motion, and the sparseness of the gene network, is it possible to estimate the parameters of the network with a greater accuracy, as also shown in Fig. where we plot the best estimates $A_{ij}^*$ obtained by both methods versus their true values $A_{ij}$: in the case of perfect retrieval all of the points should lie on the $y = x$ line.
FIG. 4: Dependence of $\eta_A$ on the fraction of priors, as obtained by analyzing one or two time-series. The scoring parameter $\eta_A$ decreases as the number of priors increases, indicating that a better estimate of the gene network $A$ is recovered.

3. Example 2: multiple time series

There are two major problems encountered when trying to infer a gene network via the analysis of time-series data. The first one is that there are usually too few time-points with respect to the large number of genes. The second one is associated to the fact that, when the system responds to an external perturbation, only the expression of the genes directly or indirectly linked to that perturbation changes, i.e., only a specific sub-network of the whole gene network is activated by the external perturbation. While through the study of the time-series it is possible to learn something about the regulatory role of the responding genes, nothing can be learnt about the regulatory role of the non-responding genes.

These problems can be addressed by using gene network retrieval procedures which are able to simultaneously analyze different time-series [Wang et al. 2006], particularly if these measure the response of the system to different perturbations, as we expect different perturbations to activate different genes. Our reverse engineering approach naturally exploits the presence of multiple time series by requiring the minimization of Eq. (7).

Here we study the network discussed in the previous example by adding to the time-series shown in Fig. 4 other ones generated by the application of two different perturbations. For sake of simplicity all time-series are measured at equally-spaced time-points, but with an elapsing time between two consecutive data points depending on the particular time-series. Hence that the problem cannot be reduced to the one of a single average time-series by exploiting the linearity of Eq. (1).

As the number of time-series increases, our determination of the gene network $A$ becomes more and more accurate. For instance, while by means of a single perturbation we obtain $\eta_A = 0.76$ ($\eta_B_0 = 0.005$), by using two time-series we obtain $\eta_A = 0.25$ ($\eta_B_0 = 0.004, \eta_B_1 = 0.003$), and by using three time series we get $\eta_A = 0.13$ ($\eta_B_0 = 0.004, \eta_B_1 = 0.002, \eta_B_2 = 0.002$).

4. Example 3: biological priors

As the traditional approach to research in Molecular Biology has been an inherently local one, examining and collecting data on a single gene or a few genes, there are now couples of gene which are known to interact in a specific way, or do not interact at all. This information is nowadays easily available by consulting public databases such as Gene Ontology. Here we show that it is possible to integrate this non-analytical information in our reverse engineering approach, improving the accuracy of the retrieved network. To this end we consider again the gene network $A$ but we introduce some constraints on a fraction $f$ of randomly selected elements of the matrices $A$ and $B$, namely $10% \leq f \leq 40%$. As our retrieval procedure tries to exchange vanishing and not vanishing elements of $A$ and $B$ we introduce the constraints as follows: if the element is zero in the exact matrices then we set it to zero and we never try to set it to a non-zero value; on the contrary, if the element is different from zero, its value is free to change and we never try to set it to zero. By using this approach we assure that our best estimates of $A$ and $B$ are
consistent with the previous knowledge. In order to stress the greater improvement that can be obtained via the use of biological priors, we consider now the same gene network $A$ and perturbations of examples 1 and 2, but we corrupt the noiseless dataset by adding a noise (see Eq. (10)) characterized by $p = 0.1$, and not by $p = 0.05$ as before. Due to the high value of the noise the linear algebraic approach is not more able to recover the gene network matrix, as it obtains a score $\eta_A = 4.40$.

We show in Fig. 4 the dependence of $\eta_A$ on the fraction of randomly selected elements of $A$ and $B$ fixed either to zero or to non-zero, both for the case in which only one or two perturbations have been used in the retrieval procedure. As expected, $\eta_A$ decreases as the number of priors increases, showing that as more biological knowledge on the system of interest is available the reliability of our reverse engineering approach improves.

5. Results on Escherichia Coli

We applied our algorithm to a nine gene network, part of the SOS network in E. Coli. The genes are recA, lexA, Ssb, recF, dinI, umuDC, rpoD, rpoH, rpoS, and the used time-series consists of six time measurements (in triplicate) of the expression level of these genes following treatment with Norfloxacin, a known antibiotic that acts by damaging the DNA. The time series is the same used in Ref. (Bansal et al. 2006), and experimental details can be found there.

Given $N_g = 9$ there are 90 unknowns to be determined, as $A$ is a $N_g \times N_g$ matrix, and $B$ is a vector of length $N_g$. Since $N_t = 6$, the experimental data allows for the writing of $N_g(N_g - 1) = 45$ equations, and for the determination of only 45 unknowns, while a literature survey (Bansal et al. 2006) suggests that there are at least 52 connections between the considered genes (including the self-feedback). As in previous works, we are therefore forced to use an interpolation technique to add new time measurements, creating a time series with 11 time points.

When applied to this dataset, our algorithm found that $\chi^2_{\text{red}}$ is minimized by a matrix $A$ with 57 not vanishing entries, and a vector $B$ with 6 non-zero elements, which are given in Table I. In the literature, there are 52 known connections between the nine considered genes, including the self-feedback. As in previous works, we are able to find 37 of these connections. Regarding the interaction with Norfloxacin, our algorithm found that primary target is $\text{recA}$, as expected.

| recA | lexA | Ssb | recF | dinI | umuDC | rpoD | rpoH | rpoS | B |
|------|------|-----|------|------|-------|------|------|------|---|
| -1.68 | -0.36 | 1.81 | 1.05 | 0.84 | - | - | -0.59 | 0.71 |
| -0.11 | -1.56 | 0.59 | 0.58 | 0.40 | -0.34 | - | - | 0.13 |
| -0.47 | 1.82 | -2.83 | -0.60 | -0.96 | -1.71 | 1.29 | - | - |
| 0.68 | 0.42 | -0.93 | -0.52 | -0.40 | -0.30 | 1.13 | - | 0.38 |
| 1.18 | 0.72 | 0.39 | -0.96 | -1.71 | 0.42 | - | - | 0.34 |
| 0.47 | -0.63 | -0.39 | -0.64 | 0.19 | -0.65 | 0.11 | - | 0.53 | - |
| -0.06 | -0.28 | 0.36 | - | -0.22 | - | - | - | 0.40 |
| - | - | -1.10 | 1.60 | -0.32 | 0.92 | -3.46 | 1.46 | - |
| -0.39 | -0.43 | - | 0.18 | 0.92 | 0.26 | 0.82 | -0.72 | -0.11 |

**TABLE I**: The matrix $A$ encoding the SOS network for E. coli: each element codes the effect of the gene of the column on the gene of the row. The last column shows the effect of Norfloxacin on the considered genes, $B$. All elements are expressed in $10^2 s^{-1}$. The matrix $A$ has 57 not vanishing elements, while $B$ has 6 non-zero elements. For visualization purposes zero elements have been replaced by a dash ‘-‘.
IV. CONCLUSIONS

In the framework of a linear deterministic description of the time evolution of gene expression levels, we have presented a reverse engineering approach for the determination of gene networks. This approach, based on the analysis of one or more time-series data, exploits the time-reversibility of the equation of motion of the system, the sparsity of the gene network and previous biological knowledge about the existence/absence of connections between genes. By taking into account this information the algorithm significantly improves the level of confidence in the determination of the gene network over previous works.

The drawback of our procedure is the computational cost, which at the moment limits the applicability of the algorithm to a small number of genes/clusters. There are two time-consuming procedures. One is the transformation of the continuous matrix $A$ in the discrete matrix $A$, which we have been avoided by using the bilinear transformation, but whose validity breaks down as the time interval between two consecutive measurements increases. The second one, which at the moment is the most expensive in time, is the computation of the inverse matrix $A^{-1}$, which we accomplish through the so-called LU decomposition whose computational cost is $O(N^3)$. Alternative methods for exploiting the reversibility of the dynamics should therefore be devised for applications with a larger number of genes.

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