Inference of gene regulatory networks and compound mode of action from time course gene expression profiles

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ABSTRACT

Motivation: Time series expression experiments are an increasingly popular method for studying a wide range of biological systems. Here we developed an algorithm that can infer the local network of gene–gene interactions surrounding a gene of interest. This is achieved by a perturbation of the gene of interest and subsequently measuring the gene expression profiles at multiple time points. We applied this algorithm to computer simulated data and to experimental data on a nine gene network in Escherichia coli.

Results: In this paper we show that it is possible to recover the gene regulatory network from a time series data of gene expression following a perturbation to the cell. We show this both on simulated data and on a nine gene subnetwork part of the DNA-damage response pathway (SOS pathway) in the bacteria E. coli.

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1 INTRODUCTION

Recent developments in large-scale genomic technologies, such as DNA microarrays and mass spectroscopy have made the analysis of gene networks more feasible. However, it is not obvious how the data acquired through such methods can be assembled into unambiguous and predictive models of these networks. Different experimental and computational methods have been proposed to tackle the network identification problem (Tong et al., 2002; Lee et al., 2002; Ideker et al., 2001; Davidson et al., 2002; Arkin et al., 1997; Yeung et al., 2002). Although implemented with some success, they are data intensive and they may require a certain degree of a priori information.

A variety of mathematical models can be used to describe genetic networks (de Jong, 2002; Savageau, 2001; Levchenko and Iglesias, 2002), including Boolean logic (Shmulevich et al., 2002; Liang et al., 1998), Bayesian networks (Hartemink et al., 2002), graph theory (Wagner, 2001) and ordinary differential equations (Tegner et al., 2003). We concentrated our efforts on the last method as it offers a description of the network as a continuous time dynamical system that can be used to infer the genes with the major regulatory functions in the network.

In a recent study (Gardner et al., 2003), we developed an algorithm (Network Identification by multiple regression—NIR) that used a series of steady state RNA expression measurements, following transcriptional perturbations, to construct a model of a nine gene network that is a part of the larger SOS network in E. coli (Gardner et al., 2003). Though the NIR method proved highly effective in inferring small microbial gene networks, it requires prior knowledge of which genes are involved in the network of interest, and the perturbation of all the genes in the network via the construction of appropriate episomal plasmids. In addition, it requires the measurement of gene expressions at steady state (i.e. constant physiological conditions) after the perturbation. This experimental setup is challenging for large networks, it is not easily applicable to higher organisms, and, most importantly, it is not applicable if there is no prior knowledge of the genes belonging to the network.

In this paper we are presenting an algorithm TSNI (Time Series Network Identification) that can infer the local network of gene–gene interactions surrounding a gene of interest by perturbing only one of the genes in the network. To this end, we need to measure gene expression profiles at multiple time points following perturbation of the gene, or genes, of interest.

We investigated the effect of noise and a limited number of data points on the performance of the algorithm, and devised techniques to overcome these problems.

Our algorithm is illustrated and tested in silico on computer simulated gene expression data and applied to an experimental gene expression data set obtained by perturbing the SOS system in the bacteria E. coli.

The novelty of our approach is in the idea of a gene-centric inference method that can be applied to infer the regulatory interactions of a gene of interest. State-of-the-art inference algorithms start from the assumption that a gene network is unknown and experiments are performed to perturb it. Gene expression data are then used to reconstruct the network. In a real life situation, large-scale gene expression data from a given cell type involve thousands of responsive genes and there are many different regulatory networks activated at the same time by the perturbations. In this case, inference methods can be successful but only on a subset of the genes (i.e. a specific network) (Basso et al., 2005). This subset of genes (network), however, cannot be defined a priori but depends on the data set. Networks involving genes that never change in the dataset cannot be inferred. We aim at developing an integrated experimental and computational approach to infer the network of a specific gene of interest.

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2 METHODS

2.1 Network model description
Our model is based on relating the changes in gene transcript concentration to each other and to the external perturbation (as shown in Fig. 1). By external perturbation we mean an experimental treatment that can alter the transcription rate of the genes in the cell. An example of perturbation is the treatment with a chemical compound, or a genetic perturbation involving overexpression or downregulation of particular genes. We use the following system of ordinary differential equations (de Jong, 2002) to represent the rate of synthesis of a transcript as a function of the concentrations of every other transcript in a cell and the external perturbation:

\[ x_i(t_k) = \sum_{j=1}^N a_{ij} x_j(t_k) + \sum_{l=1}^P b_{il} n_l(t_k), \]

where \( i = 1 \ldots N, k = 1 \ldots M, x_i(t_k) \) is concentration of transcript \( i \) measured at time \( t_k; x_j(t_k) \) is the rate of change of concentration of gene transcript \( i \) at time \( t_k \); \( a_{ij} \) represents the influence of gene \( j \) on gene \( i \) with a positive, zero or negative sign indicating activation, no interaction and repression, respectively, \( b_{il} \) represents the effect of \( l \)-th perturbation on \( x_i \) and \( n_l(t_k) \) represents the \( l \)-th external perturbation at time \( t_k \).

Equation (1) at time \( t_k \) can be rewritten in a more compact form using matrix notation

\[ X(t_k) = A X(t_k) + B U(t_k), \]

where \( X(t_k) \) is an \( N \times 1 \) vector of mRNA concentration of \( N \) genes at time \( t_k; X(t_k) \) is a \( N \times 1 \) vector of the first derivatives of \( X(t_k) \) at time \( t_k \); \( A \) is a \( N \times N \) connectivity matrix, composed of elements \( a_{ij} \); \( B \) is a \( N \times P \) matrix representing the effect of \( P \) perturbations on \( N \) genes, \( U(t_k) \) is a \( P \times 1 \) vector representing \( P \) perturbations at time \( t_k \) and \( M \) is the number of time points \( t_k \) in the time series experiment. The unknowns to calculate are the connectivity matrix \( A \) and matrix \( B \). Element \((i,l)\) of \( B \) will be different from zero if the \( i \)-th gene is a direct target of the \( l \)-th perturbation.

2.2 TSNI Algorithm
The TSNI algorithm identifies the network of the genes \( A \) as well as the direct targets of the perturbations \( B \). To identify the network means to retrieve \( A \) and to identify the direct targets of the drugs means to identify \( B \), by solving Equation (2). Solving this equation is possible only if \( M \geq N+P \). Since usually we have less data points (time points) than the number of genes, we cannot solve Equation (2) directly. A solution can be found either by increasing the number of data points artificially by interpolation or by dimensional reduction techniques. In our algorithm we apply both approaches to the dataset: first, we apply a cubic smoothing spline filter with an adjustable smoothing parameter (de Boor, 2001). This smoothing filter reduces the fluctuations in the data introduced by noise. We increase the number of time points by interpolating the smoothed data using piecewise cubic spline interpolation. Third, we apply Principle Component Analysis (PCA) to the dataset in order to reduce its dimensionality and solve the equation in the reduced dimension space as described below.

To solve Equation (2) we need the first time derivative of gene expression profile. Since the data are noisy, taking derivatives will further increase the noise level. In order to avoid this problem we convert Equation (2) to its discrete form (Ljung, 1999):

\[ X(t_{k+1}) = A_d X(t_k) + B_d U(t_k), \]

where \( A_d \) is the network in the discrete space, which is different from \( A \) (Ljung, 1999) and \( B_d \) is discrete counterpart of \( B \). Rewriting Equation (3)

\[ X(t_{k+1}) = [A_d \ B_d] \begin{bmatrix} X(t_k) \\ U(t_k) \end{bmatrix}, \]

which can be written for all time points;

\[ X = H \ast Y, \]

where

\[ H = [A_d \ B_d] \]

and

\[ Y = \begin{bmatrix} X \\ U \end{bmatrix}. \]

Dimensions of \( X, U, H \) and \( Y \) are \( N \times (M-1), P \times (M-1), N \times (N+P) \) and \( (N+P) \times (M-1) \), respectively. We apply the PCA to reduce the dimension of Equation (5) by decomposing \( Y \) using singular value decomposition (Lay, 2002):

\[ X = H \ast V \ast D \ast T', \]

where columns \( V \) are left singular vectors, rows of \( T' \) are right singular vectors and \( D \) is a diagonal matrix of singular values arranged in descending order. Choosing the top \( k \) singular values, we can write

\[ X = Z_d \ast Y_R, \]

where \( Z_d \) is obtained by taking first \( k \) columns of \( H \ast V \) and \( Y_R \) is the data in the reduced dimension obtained by taking the first \( k \) rows of \( D \ast T' \). The solution is obtained by taking pseudo-inverse of \( Y \) to obtain

\[ Z_d = X \ast Y_R^T \ast (Y_R \ast Y_R^T)^{-1}. \]

We then project the solution \( Z_d \) obtained in the reduced dimension space to the original dimension space using matrix \( V \) (Montgomery et al., 2001) to get \( A_{d'} \) and \( B_{d'} \) in order to compute the continuous network model \( A \) and continuous form of \( B \) from its discretized form \( A_d \) and \( B_d \) respectively, we apply the following bilinear transformation (Ljung, 1999):

\[ A = \frac{2A_d - I}{\delta t A_d + I} \]

\[ B = (Ad + I)^{-1} \ast A \ast B_d. \]
where \( I \) is the square identity matrix of dimension \( N \times N \) and \( \delta t \) is the sampling interval. The transformation from discrete to continuous model is an important step. All the work presented in the literature till now is based on the discrete time model even if the dynamics of gene regulation is continuous in time.

### 2.3 Simulated gene expression data

Before applying the algorithm to the real dataset, we tested its performance on simulated datasets. We tested the performance on two different simulated datasets, one corresponding to a set of small gene networks with 10 genes and another one corresponding to larger networks with 1000 genes. To test the performance of TSNI on both these networks with 10 and 1000 genes, we generated 100 random networks with an average of 5 and 100 connections per gene, respectively. Each network was represented by a full rank sparse matrix \( A (N \times N) \) with eigenvalues with a real part less than 0 (Ljung, 1999) to ensure the stability of the dynamical systems, i.e. all the gene mRNAs reach an equilibrium between their transcription rate and degradation rate after a given time period. We applied \( P = 1 \) perturbations to each network. For networks of 10 genes, we perturbed 1 gene, while for networks of 1000 genes, we performed two sets of perturbations, one in which we perturbed only 1 gene and the other in which we perturbed 100 genes simultaneously. The information of which gene is perturbed is contained in \( B (N \times 1) \). \( B \) has all its elements equal to 0 except for the genes that are the direct target of the perturbation. \( U (1 \times M) \) contains the information about what kind of perturbation is applied. In our simulation we applied a constant perturbation, so all elements of \( U \) are kept constant (1 in our simulation).

The simulated gene expression profile dataset \( X = [X(t_1) \ldots X(t_{N})] \) was obtained using \textit{lsim} command in MATLAB [see supplementary of Gardner et al. (2003) for more details of how to obtain simulated gene expression profile] by solving

\[
X = AX + BU,
\]

where \( X (N \times M) \) is the response of the genes at \( M \) time points following the perturbation. The end time \( t_e \), of the simulated time series was chosen equal to four times the inverse of the real part of the smallest eigen value of \( A \) (Ljung, 1999). This ensures that at time \( t_e \), all the genes are close to their steady-state values. We then selected 5 and 10 time points for the 10 and 1000 gene networks, respectively. These time points were equally spaced from the start time \( t_0 \) to the end time \( t_e \). White Gaussian noise was added to the data matrix with zero mean and varying the standard deviation from \( \sigma = 0 \) to \( \sigma = 50 \times |l||x| \), with an interval of 0.1, where \( |l||x| \) represents the absolute values of the elements of \( X \) (Gardner et al., 2003). The simulated gene expression time courses are then filtered using the smoothing algorithm described in (de Boer, 2001) with a default parameter of 0.8. Smoothing is widely used in signal processing to remove outliers, if any, from the time course, to reduce the measurement noise, and, to increase the number of data points via interpolation. However, to our knowledge, it has never been applied on time series gene expression data.

#### 2.3.1 Assessing the performance of the algorithm.

- **Gene regulatory network:** Matrix \( A \) inferred by the TSNI algorithm has \( N \times N \) elements describing the regulatory influences among the \( N \) genes in the network. In the recovered network all the elements are non-zero. To make the network sparse (Gardner et al., 2003), we set the smallest \( h \) elements in \( A \) to zero. We calculated the ratio, \( r_c \), of number of correctly identified zero coefficients in the recovered \( A \) to the number of zero elements in the original \( A \) and the ratio, \( r_{nc} \), of total number of non-zero elements in the recovered \( A \) whose signs are in agreement with the signs of non zero elements in the original \( A \). We varied \( h \) from 0 to the number of elements in \( A \), and calculated \( r_c \) and \( r_{nc} \), for each \( h \).

- **Direct targets of the perturbations:** Matrix \( B \) inferred by the algorithm has \( N \times 1 \) elements that describe the direct targets of the perturbation.

In the recovered \( B \), all the elements are non-zero. We sort all the elements of \( B \) according to their absolute values and selected the top \( h \) largest elements and set remaining \( N - h \) elements to zero. Once the \( N - h \) elements of \( B \) are set to 0, to assess how well the algorithm can infer the direct targets of perturbation, we defined as True Positives (TP) those elements \( b_i \) of the inferred \( B \) that are different from 0 and that are non-zero in the original \( B \). Similarly False Positives (FP) are all the elements \( b_i \) that are different from 0 while the original \( b_i \) are 0. Analogously, we defined the False Negatives (FN) and True Negatives. We measured the overall performance by computing the positive predictive value (PPV) \( \frac{TP}{TP+FP} \) and sensitivity \( \frac{TP}{TP+FN} \) by varying \( h \) from 1 to \( N \).

### 2.4 Experimental methods

#### 2.4.1 Growth conditions and E. coli treatment

The bacterial strain MG1655 was grown over night in 5 ml LB amp 100 \( \mu \)g/ml with shaking (300 r.p.m.) at 37°C. The time course experiment consisted in the induction with 10 \( \mu \)g/ml of Norflaxacin and extraction of the total RNA at the following time points: 0, 12, 24, 36, 48 and 60 min from the drug treatment. Each experiment was done in triplicate; positive controls were done at 24 and 60 min from the induction with Norflaxcin.

#### 2.4.2 Preparation of E. coli for hybridization to Affymetrix Chips

- **RNA extraction:** Cultures were centrifuged at 3000 r.p.m. for 5 min at 4°C, the pellets were re-suspended in RNA protect and incubated for 10 min at room temperature. The RNA protect is completely poured off and the cells pellets were frozen at −80°C. RNA was prepared using the spin protocol for the RNAeasy 96 kit (Qiagen on Column Dnase digestion).

- **cDNA synthesis:** For each sample reverse transcription of RNA was performed using First Strand cDNA kit according to manufacturer’s instructions. The reactions were degraded by the addition of 1 M NaOH and heating to 65°C for 30 min. The reactions were neutralized with 1 M HCl. The reactions were purified using Qiagen QIAquick columns following the manufacturer’s protocol. The DNA was eluted from the columns with 40 \( \mu \)l of EB buffer. To digest any genomic DNA present in the samples, 3 \( \mu \)g of each cDNA was fragmented by combining with the following 10x One-Phor-All Buffer, 1 \( \mu \)l DNase I, in a final volume of 50 \( \mu \)l H2O. The reactions were incubated in a thermocycler at 37°C for 10 min without the hot top, followed by inactivation of the DNase I at 98°C for 10 min.

- **cDNA labeling and hybridization:** The fragmented cDNAs were end-labeled using an Enzo BioArray Terminal Labeling Kit with Biotin-ddUTP. To each tube of fragmented cDNA the following was added: 5x reaction buffer, 10x: CoCl2, 100x: Biotin-ddUTP, 50x Terminal Deoxynucleotide Transferase in a final volume of 100 \( \mu \)l H2O. The reactions were incubated in a heatblock at 37°C for 1 h 15 min. The reactions were quenched with the addition of 2 \( \mu \)l 0.5 M EDTA, pH 8.0, according to manufacturer instructions. A 3 \( \mu \)l aliquot was taken from each tube and dried. A 2 \( \mu \)g/ml NeutrAvidin was added to each tube and incubated at room temp for 5 min. The conjugates along with an additional 3 \( \mu \)l of the labeled cDNA were electrophoresed on 3% non-denaturing agarose, 1x TAE gels at 250 V for 20 min. The gels were stained with a solution of 0.1% SYBR Gold in 1x TEA for 25 min then imaged. The fragmented, labeled cDNAs were prepared for hybridization by combining with the following: cDNA, 2x Hybridization buffer, 50 mg/ml acetylated BSA, 10 mg/ml Herring Sperm DNA, 3 nM Control Oligo B2, Agent-X in a total volume of 200 \( \mu \)l. The mixtures were loaded on each chip and hybridized overnight at 45°C and 60 r.p.m. Following a
minimum of 15 h of hybridization, the chips were stained and scanned according to Affymetrix protocols.

2.4.3 Microarray data analysis. We processed the microarray data using the rma command of the Bioconductor package that performs normalization and gene expression estimation from replicates using the algorithm described by Gautier et al. (2004). By calculating the mean \( \mu \) and standard deviation \( \sigma \) on the three replicates of each time point, we found that the noise level in our experiment is \( \frac{\sigma}{\mu} \approx 13\% \) (\( \mu = 0.13 \)).

3 RESULTS

3.1 Choosing the parameters of the model

In order to set the best value for the number of interpolated points and the number of principle components, we applied the TSNI algorithm to each simulated dataset and varied: (1a) the number of interpolated data point ranging from 0 (no interpolation) to 10\( \times M \) (10 times the number of experimental points in the time series); (2b) the number of principle components from 1 to \( \min(N, M) \) (minimum of the number of genes in the network or the number of time points). For each of these parameters, we calculated the average of \( r_{nc} \) vs \( r_z \) across 100 random systems by varying \( h \) from 0 to the number of elements in \( A \) and plotted \( r_{nc} \) vs \( r_z \). Ideally, when we increase the number of nonzero elements in \( A \) by varying \( h \), we should start identifying non-zero elements correctly with zero false positives, which will keep the value of \( r_z \) equal to 1 and increase \( r_{nc} \) from 0 to 1. The best value of \( h \) will correspond to that value where both \( r_z \) and \( r_{nc} \) equal one. At the point when \( A \) is fully connected we should have \( r_{nc} \) equals 1 and \( r_z \) equals zero. We selected as the best set of parameters, the ones that gave the maximum area under the \( r_{nc} \) versus \( r_z \) curve (since we have one of such curves for each parameter value that we explored).

3.2 Result on simulated 10 gene network

To select the best set of parameters, we plotted the area under \( r_{nc} \) versus \( r_z \) curve for all the set of parameters. First we plotted the area under \( r_{nc} \) versus \( r_z \) curve for different interpolation levels and different principle components for various noise levels (see Figure 1S for 0% noise level and Figure 2S for 10% noise level in supplementary). These plots shows that double interpolation (two times the number of data points in the time series) works best. Once the interpolation is decided, we then plotted the area under \( r_{nc} \) versus \( r_z \) for different noise level and different principle components (see fig 3S in supplementary) after fixing the interpolation to two times. From this we found that if the noise level is very low, then three principle components work best. At 10% noise level, two principle components work better, whereas at higher noise levels only one principle component works well. This is to be expected, since higher principle components capture also the noise signal, whereas most of the information is captured in lower components. We therefore selected double interpolation and two and three principle components for our study, as we know that the noise level in our real data is \( \approx 13\% \) (see Section 2.4.3).

Figure 2 shows the plot for average of \( r_{nc} \) versus \( r_z \) across 100 random networks for double interpolation and three principle components at various noise level. The dash dotted line shows the performance when we select the connections in the network randomly. The performance of the algorithm decreases clearly...
with increasing noise levels. We also checked how well we can
recover $B$ (graph not shown here) and the result shows that we can
predict the targets of the perturbation with a sensitivity of 99% with
96% PPV, i.e. 96% of the times we were able to tell correctly the
correct target without any false positives.

3.3 Result on simulated 1000 gene network

3.3.1 Results on inferring network $(A)$

On larger networks of 1000 genes the performance of our algorithm in recovering the $A$ matrix is not very good and in fact $r_{cc}$ versus $r_c$ curve overlapped the random curve. This happened because the network is not fully observable and one experiment does not yield sufficient information to infer the network. To check whether we can infer the local network around a gene of interest, we selected the column corresponding to the perturbed gene in the simulation in which we perturbed only one gene, and compared it with the corresponding column in the original network $A$. This corresponds to infer the genes that are directly regulated by the perturbed gene. In Figure 3 we computed the PPV and sensitivity in the same way as described above when assessing the performance in getting the direct targets of the perturbation (Section 2.3.1). We found that for double interpolation and 1 principle components (which is found again by the checking the set of parameters which gives the maximum area under ppv vs sensitivity curve for all set of parameters) at 10% noise we get 75% sensitivity with almost 100% PPV. PPV decreases to 90%

3.4 Results on $E. coli$

3.4.1 Results on inferring the network $(A)$

We applied our TSNI algorithm to a nine gene network, part of SOS network in $E. coli$. Genes are the same as the ones we used in our previous work (Gardner et al., 2003) in order to see how well we can replicate the results. To this end we computed the average of the three replicates for each time point following treatment with Norfloxacin, a

Fig. 3. Plot of PPV versus sensitivity for 1000 genes network when predicting genes directly regulated by the perturbed gene (column of $A$). Two different curves for 10 and 50% noise level are shown. The dash dotted horizontal line at the bottom shows the curve when we select the genes randomly. In the inset, is the PPV versus sensitivity curve for the prediction of targets of perturbation for 1000 gene network ($B$).
known antibiotic that acts by damaging the DNA. In order to assess the performance of the algorithm on this experimental data, we compared the inferred network with the one we identified in our previous work (Gardner et al., 2003) and with a literature survey of the known interactions among these nine genes (Fig. 4). We found 43 connections, apart from the self-feedback, between these genes that are known in literature. The network obtained by the algorithm for the E. coli time-series data for three principle components and double interpolation is shown in Table 1. We compared this predicted network with known connections from the literature and plotted rnc versus r. The cross on the plot shows the value of r, which is obtained by comparing the network predicted in our previous work (Gardner et al., 2003) with the network from the literature. NIR found 22 connections correctly out of 43 known connections. The result of our present study is similar to our previous work, even if we used only a single perturbation experiment and 5 time points as compared to our previous work in which we used nine different perturbation experiments and we also assumed the matrix B to be known.

When we used the information that there should be five connections for each gene, [from our previous work (Gardner et al. (2003))], and set four elements in each row of the inferred matrix $A$ to zero, then our algorithm finds 20 connections correctly (diamond in Fig. 5).

### 3.4.2 Results on inferring the targets (B)

To check the prediction of B, we considered the treatment of E.coli with Norfloxacin equivalent to the a perturbation to recA. Norfloxacin is a member of fluoroquinolone class of antimicrobial agents that target the prokaryotic type II topoisomerase II (DNA gyrase) and topoisomerase IV inducing the formation of single-stranded DNA and thus activating the SOS pathway via activation of the recAp protein. Quinolones have been previously demonstrated to induce recA and other SOS-responsive genes in E.coli. (Phillips et al., 1987). We checked that we can get recA as the strongest target. This gives 100% value for both positive predicted value and sensitivity, which shows that TSNI algorithm is very good in predicting drug target, at least for small network.

We then checked how well we can do if we select a larger dataset of genes in E.coli. We applied our algorithm to the 300 genes which statistically responded to the Norfloxacin treatment. We ordered the absolute value of all elements in recovered $B$ by TSNI and looked at the top 50 genes (Table 1S in supplementary). We found that recA was ranked 14 and there were 9 genes which belong to SOS pathway in the top 50 genes.

### 3.5 Comparison with Dynamic Bayesian Network

We then compared our algorithm with Dynamic Bayesian Network (DBN), one of the most successful algorithms available now for time series. The standard DBN (Murphy, 2001) cannot be compared with our algorithm directly, since DBN infers an undirected network (i.e. it does not give the sign of the connection in the network), and it is not able to infer feedback loops. We therefore decided to compare our work with the work by Yu et al. (2004). In this paper, the authors developed a generalization of the DBN algorithm to infer directed networks with feedback loops. The authors then applied their algorithm on a network of 20 genes and tested its performance by using different number of data points, ranging from 25 to 5000. Figure 5a in their paper shows that for high number of data points (>1000), they are able to recover 98% of the connections in the network correctly, but using lower number number of data points, their performance quickly decreases. In addition, their performance varies a lot, depending on the number of parents of each node. When the network is very sparse, i.e. each node has few parents (1 or 2) it works well, but when the network becomes dense, with three or more parents per node then the performance decreases a lot. In our dataset, we assumed only 5 data points for a 10 gene network. In addition, we assume that the network is not very sparse and has five parents for each node (Gardner et al., 2003). For this dataset, therefore, the performance of the algorithm of Yu et al. (2004) is equivalent to the random algorithm.

### 4 DISCUSSION

In this pilot study we investigated the possibility of inferring the local network of regulatory interactions surrounding a gene of interest when there is no a priori knowledge of the genes belonging to network, nor about the structure of the network. We found that by perturbing a gene of interest and measuring the response of the genes following the perturbation, it is possible to partially reconstruct the regulatory network. Our approach confirmed many of the known information from literature about different interactions in the SOS network. We propose a robust method that allows to infer a continuous-time model of a gene network from time series data without requiring the estimation of the first derivatives, thanks to the use of the bilinear transformation. We also show that smoothing is a very powerful technique to reduce the noise in the data and should be used prior to interpolation.

The TSNI algorithm could be a powerful methodology for the drug discovery process since it would be able to identify the compound mode of action via a time-course gene expression profile. We have already shown in a recent study (di Bernardo et al., 2005), using a different approach that requires large collection of whole-genome gene expression profiles in yeast, that it is possible to infer compound mode of action by analyzing transcriptional response.
even when the compound does not directly affect the transcriptional response.

Our model is scalable to large networks, thanks to the dimensional reduction and smoothing and interpolation, and to the reduced number of experiments it requires, since only one perturbation experiment is necessary. We are currently applying the algorithm to infer a network from whole genome time series microarrays in mammalian primary cells.

There are two main innovative aspects in our algorithm: (1) we propose an experimental and computational methodology to infer the gene regulatory network from time expression data in which a specific gene of interest is present. This can be achieved either by directly perturbing the gene using an inducible vector for its overexpression or downregulation, or through a compound known to activate the pathway of interest (i.e. Norfloxacin to activate the SOS-pathway as shown in this paper); (2) the approach can be used to speed up the drug discovery process, in that, it is able to predict for an unknown compound, its direct molecular targets from gene expression data following treatment. Importantly, our algorithm requires a limited amount of data as compared with Dynamic Bayesian network and Bayesian network. These methods are very powerful when large number of data points are available. In addition, to our knowledge, DBN and BN have never been applied to...
infer the targets of a compound or of perturbation from gene expression data.

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