

# Inferring slowly-changing dynamic gene-regulatory networks

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## Abstract

Dynamic gene-regulatory networks are complex since the interaction patterns between its components mean that it is impossible to study parts of the network in separation. This holistic character of gene-regulatory networks poses a real challenge to any type of modelling. Graphical models are a class of models that connect the network with a conditional independence relationships between the random variables. By interpreting the random variables as gene activities and the conditional independence relationships as functional non-relatedness, graphical models have been used to describe gene-regulatory networks. Whereas the literature has been focused on static networks, most time-course experiments are designed in order to tease out temporal changes in the underlying networks. It is typically reasonable to assume that changes in genomic networks are few, because biological systems tend to be stable.

We introduce a new model for estimating slowly changes in dynamic gene-regulatory networks, which is suitable for high-dimensional data, e.g. time-course genomic data. Our aim is to estimate a dynamically changing genomic network based on temporal activity measurements of the genes in the network. Our method is based on the penalized likelihood with  $\ell_1$ -norm, that penalizes conditional dependencies between genes as well as differences between conditional independence elements across time points. We also present a the heuristic search strategy to find optimal tuning parameters. We re-write the penalized maximum likelihood problem into a standard convex optimization problem subject to linear equality constraints. We show that our method performs well in simulation studies. Finally, we apply the proposed model to a time-course T-cell dataset.

**Keywords:** gene-regulatory networks; graphical models;  $L_1$  penalized inference

## 1 Introduction

A single microarray experiment provides a snapshot of the expression of many genes simultaneously under a particular condition. Gene expression is a temporal process, in which different genes are required and synthesized for different functions and under different conditions. Even under stable conditions, due to the continuous degradation of proteins, mRNA is transcribed continuously and new proteins are generated. This process is highly regulated. In many cases, the expression programme is initiated by the activation of a few transcription factors, which in turn activate many other genes that react in response to the newly arisen conditions. Transcription factors are proteins that bind to specific DNA sequences, thereby controlling the flow of genetic information from DNA to mRNA. For example, when cells are faced with a new external environment, such as starvation [1], infection [2] or stress [3], they react by activating a particular expression program. Taking a snapshot of the expression profile following a new condition can reveal some of the genes that are specifically expressed under the new condition. However, in order to discover the interaction between these genes, it is necessary to measure the genes across time in a time-course expression experiment. These temporal measurements potentially allow us to determine not only the stable state following a new condition, but also the gene interactions that were activated in order to arrive at this new state. The biological and computational issues involved in designing and analyzing gene expression data in general, and time-course expression data in particular, is discussed in [4].

In this paper, we propose a graphical model for describing the temporal interaction patterns between genes. Graphical models explore conditional independence relationships between random variables. They can be divided into directed graphical models, e.g. Bayesian networks [5, 6], undirected graphical models, e.g. Gaussian graphical models [7, 8] and mixed versions, such as chain graphical models [7]. Bayesian networks have been successfully used to describe certain types of gene regulatory networks [9]. However, Bayesian networks suffer two major limitations. Firstly, they cannot be used to describe cyclic graphs. This rules out using them for describing simultaneous feedback loops in gene regulatory networks. Secondly, they perform poorly on sparse microarray data as shown by [10]. It is possible to “unroll” cycles into spirals through time, so the first limitation can partially be

<sup>1</sup>overcome [11–13]. Instead, we propose to model such cycles more directly as undi-<sup>1</sup>  
<sup>2</sup>rected edges in our conditional independence graph. Furthermore, our method will<sup>2</sup>  
<sup>3</sup>allow for “directed” edges between consecutive time points. 3

<sup>4</sup> The class of Gaussian graphical models (GGM) have been particularly popu-<sup>4</sup>  
<sup>5</sup>lar. The main advantage for GGMs is that the precision matrix, i.e. the inverse<sup>5</sup>  
<sup>6</sup>of the covariance matrix, can be used to “read off” the conditional independence<sup>6</sup>  
<sup>7</sup>relationships between the random variables. The literature on estimating the in-<sup>7</sup>  
<sup>8</sup>verse covariance matrix goes back to [14], who also introduced hypothesis testing<sup>8</sup>  
<sup>9</sup>approaches to determining whether particular elements of the inverse covariance<sup>9</sup>  
<sup>10</sup>matrix are zero. The more zeroes in the inverse covariance matrix, the sparser the<sup>10</sup>  
<sup>11</sup>underlying conditional independence graph. 11

<sup>12</sup> Regulatory elements in genetic networks are highly structured. In order to guar-<sup>12</sup>  
<sup>13</sup>antee an appropriate response to a particular change in the environment, most gene<sup>13</sup>  
<sup>14</sup>interactions are highly specific. The detailed molecular structure of genes and gene<sup>14</sup>  
<sup>15</sup>products are responsible for this level of specificity. Another biological requirement<sup>15</sup>  
<sup>16</sup>is that gene regulation is fast in reacting to changes in the environment. Heat shocks<sup>16</sup>  
<sup>17</sup>should almost instantaneously result in an adaptive response from the yeast cell.<sup>17</sup>  
<sup>18</sup>From this point of view signals should be able to travel fast through the gene reg-<sup>18</sup>  
<sup>19</sup>ulatory network: the network should have a small world property. Consequently,<sup>19</sup>  
<sup>20</sup>most gene regulatory networks are sparse small-world graphs. If the expression of<sup>20</sup>  
<sup>21</sup>the genes can be assumed to be normally distributed, then this means that most of<sup>21</sup>  
<sup>22</sup>the elements in the precision matrix are equal to zero. A standard approach in sta-<sup>22</sup>  
<sup>23</sup>tistical modelling to identify zeroes in the precision matrix is the backward stepwise<sup>23</sup>  
<sup>24</sup>selection method, which starts by removing the least significant edges from a fully<sup>24</sup>  
<sup>25</sup>connected graph, and continues removing edges until all remaining edges are signif-<sup>25</sup>  
<sup>26</sup>icant according to individual partial correlation tests. A conservative simultaneous<sup>26</sup>  
<sup>27</sup>testing procedure was proposed by [15]. However, [16] showed that this two-step<sup>27</sup>  
<sup>28</sup>procedure, in which parameter estimation and model selection are done separately,<sup>28</sup>  
<sup>29</sup>can lead to instability: small perturbations in the data can result in completely<sup>29</sup>  
<sup>30</sup>different graph structures estimates [16]. 30

<sup>31</sup> [17] showed that  $\ell_1$  penalized likelihood is a sensible way to introduce sparse<sup>31</sup>  
<sup>32</sup>solutions in a regression setting. The same idea can be used to estimate sparse<sup>32</sup>  
<sup>33</sup>Gaussian graphical models, i.e. to induce zeroes in the estimated inverse covariance<sup>33</sup>

<sup>1</sup>matrix. By penalizing the likelihood by a multiple of the  $\ell_1$ -norm of the elements<sup>1</sup>  
<sup>2</sup>of the inverse of the covariance matrix results into exact zeroes in the penalized<sup>2</sup>  
<sup>3</sup>maximum likelihood estimate for large enough values of the multiple, which is an<sup>3</sup>  
<sup>4</sup>additional tuning parameter. The larger the tuning parameter the more zeroes will<sup>4</sup>  
<sup>5</sup>be estimated in the precision matrix. [18] introduced the  $\ell_1$  penalized Gaussian<sup>5</sup>  
<sup>6</sup>graphical model. [19] showed that it is possible to select the tuning parameter<sup>6</sup>  
<sup>7</sup>in such a way as to control the familywise error rate. [20] introduced a fast and<sup>7</sup>  
<sup>8</sup>efficient algorithm to calculate the so-called graphical lasso solution. The graphical<sup>8</sup>  
<sup>9</sup>lasso estimates a single static network for a single condition. When there are multiple<sup>9</sup>  
<sup>10</sup>conditions, it may be sensible to presuppose a roughly common structure and jointly<sup>10</sup>  
<sup>11</sup>estimate common links across the graphs. [21] proposed a method that links the<sup>11</sup>  
<sup>12</sup>estimation of several graphical models through a hierarchical penalty. This graphical<sup>12</sup>  
<sup>13</sup>model leads to improvements compared to fitting separate models, since it borrows<sup>13</sup>  
<sup>14</sup>strength from other related graphs. Recently, [22] proposed a factorially coloured<sup>14</sup>  
<sup>15</sup>graph to estimate a common dynamic structure. 15

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<sup>18</sup> In this paper we propose a model to estimate slowly changing dynamic graphs<sup>18</sup>  
<sup>19</sup>using the  $\ell_1$ -regularization framework. The main idea is to impose  $\ell_1$ -norm to pe-<sup>19</sup>  
<sup>20</sup>nalize not only the inverse covariance matrix, but also changes in the inverse covari-<sup>20</sup>  
<sup>21</sup>ance matrix over time. The new method is suitable for studying high-dimensional<sup>21</sup>  
<sup>22</sup>time-course gene activity data. In order to solve the penalized maximum likelihood<sup>22</sup>  
<sup>23</sup>problem, we take advantage of an efficient solver developed by [23] to solve the opti-<sup>23</sup>  
<sup>24</sup>mization problem with linear constraints. We propose a heuristic search algorithm<sup>24</sup>  
<sup>25</sup>to fix the tuning parameters, that regulate sparsity and dynamic changes in the<sup>25</sup>  
<sup>26</sup>networks. 26

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<sup>28</sup> The rest of this paper is organized as follows. Next section gives a description of<sup>28</sup>  
<sup>29</sup>our motivating example and a brief overview of Gaussian graphical models. In Sec-<sup>29</sup>  
<sup>30</sup>tion 3, we describe the slowly changing dynamic network model and its estimation.<sup>30</sup>  
<sup>31</sup>In Section 4, we show the results of a simulation study and apply our method to<sup>31</sup>  
<sup>32</sup>the time-course T-cell dataset. Finally, we discuss the advantages of our method<sup>32</sup>  
<sup>33</sup>and point out further directions for development. 33

## <sup>1</sup>2 Motivation: T-cell activation

<sup>2</sup>T-cells are a white blood cell that play a central role in cell-mediated immunity.<sup>2</sup>  
<sup>3</sup>Activation of T-cells occurs through the simultaneous engagement of the T-cell re-<sup>3</sup>  
<sup>4</sup>ceptor and a costimulatory molecule, like CD28 or ICOS. Both are required for<sup>4</sup>  
<sup>5</sup>the production of an immune response. The signalling pathways downstream from<sup>5</sup>  
<sup>6</sup>activation usually engages the PI3K pathway and recruiting PH domain containing<sup>6</sup>  
<sup>7</sup>signaling molecules, like PDK1, that are essential for the activation of PKCtheta,<sup>7</sup>  
<sup>8</sup>and eventual IL-2 production. Although certain things are known about the struc-<sup>8</sup>  
<sup>9</sup>ture of the T-cell pathway, its timing and its precise structure are still unknown.<sup>9</sup>

<sup>10</sup>Two cDNA microarray experiments were performed to collect gene expression<sup>10</sup>  
<sup>11</sup>levels for analyzing T-cell activation. Human T-cells coming from the cell line<sup>11</sup>  
<sup>12</sup>Jakart were cultured in a laboratory. When the culture reached a consistency of<sup>12</sup>  
<sup>13</sup>10<sup>6</sup> cells/ml, the cells were treated with two treatments, the calcium ionosphere<sup>13</sup>  
<sup>14</sup>and the PKC activator phorbol ester PMA. This stimulation of the T-cells resulted<sup>14</sup>  
<sup>15</sup>in their activation. Gene expression levels for 88 genes were collected across 10 time<sup>15</sup>  
<sup>16</sup>points: the first one just before T-cell activation, at a nominal time-point 0, and 9<sup>16</sup>  
<sup>17</sup>time points at 2, 4, 6, 8, 18, 24, 32, 48, 72 hours after T-cell activation. In the first<sup>17</sup>  
<sup>18</sup>experiment the microarray was divided such that 34 sub-arrays were obtained. Each<sup>18</sup>  
<sup>19</sup>of these 34 sub-arrays contained the strands of the 88 genes under investigation.<sup>19</sup>  
<sup>20</sup>Strands are the complementary bases for the rRNA, which is the single-stranded<sup>20</sup>  
<sup>21</sup>transcribed copy of the DNA. In the second microarray experiment the microarray<sup>21</sup>  
<sup>22</sup>was divided into 10 sub-arrays. Each of these 10 sub-arrays contained the strands of<sup>22</sup>  
<sup>23</sup>the 88 genes under investigation. Both microarray experiments used ten different<sup>23</sup>  
<sup>24</sup>slides to collect the ten temporal measurements. The experiment is described in<sup>24</sup>  
<sup>25</sup>detail in [24].<sup>25</sup>

<sup>26</sup>Two further steps were conducted by [24] to obtain a set of genes that were<sup>26</sup>  
<sup>27</sup>highly expressed and normalized across the two microarray experiments. Firstly,<sup>27</sup>  
<sup>28</sup>genes with high variability between the two microarrays and within the same time<sup>28</sup>  
<sup>29</sup>point were removed. No further information is available about the minimum level<sup>29</sup>  
<sup>30</sup>of reproducibility they adopted. According to [24], thirty-one genes were to be<sup>30</sup>  
<sup>31</sup>removed since they did not show enough reproducibility. Secondly, normalization<sup>31</sup>  
<sup>32</sup>methods were applied to remove systematic variation due to experimental artifacts.<sup>32</sup>  
<sup>33</sup>The normalization method used is described in [25].<sup>33</sup>

1 At this point we assume that the 44 sub-array replicates are independent samples<sup>1</sup>  
 2 and that the temporal replicates across these sub-arrays are functionally dependent<sup>2</sup>  
 3 replicates. These two assumptions result in a dataset of 44 independent replicates<sup>3</sup>  
 4 across 57 genes and 10 time points. 4

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### 6 3 Methods 6

7 In this section, we describe the model that we adopt in order to study the under-7  
 8 lying time-varying genomic network for the T-cell data. We argue that time-course<sup>8</sup>  
 9 datasets should be analyzed in a way, that is sensitive to the underlying biology.<sup>9</sup>  
 10 If one does not use a model that is able to describe a time-varying network, there<sup>10</sup>  
 11 would not have been a point in performing a time-course experiment. The bioin-<sup>11</sup>  
 12 formatic tools should be adjusted to the needs of the biologist, who wants to infer<sup>12</sup>  
 13 particular aspects of the system. In this section, we first introduce a general graph-<sup>13</sup>  
 14 ical model. Secondly, we extend this model to the slowly changing graphical lasso<sup>14</sup>  
 15 model. Finally, we describe the computational details of performing penalized max-<sup>15</sup>  
 16 imum likelihood. 16

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#### 18 3.1 Gaussian graphical model 18

19 A graphical model is a tuple  $(G, \mathbb{P})$ , where  $G = (V, E)$  is a graph with edges  $E$ <sup>19</sup>  
 20 that describe the conditional independence relationships of probability measure<sup>20</sup>  
 21  $\mathbb{P}$  on the vertices  $V$ . This means that one can use the graph  $G$  to read off the<sup>21</sup>  
 22 functional relationships between the random variables associated with the vertices.<sup>22</sup>  
 23 In particular, for any triple  $(A, B, S)$  of disjoint subsets of  $V$  such that  $S$  separates<sup>23</sup>  
 24  $A$  from  $B$  in  $G$ , we have that for  $Y \sim \mathbb{P}$ , 24

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$$26 \quad \mathbf{Y}_A \perp_{\mathbb{P}} \mathbf{Y}_B \mid \mathbf{Y}_S. 26$$

27 This so-called global Markov property in turn implies the local and pairwise Markov<sup>27</sup>  
 28 properties. 28

29 In this paper, we will assume that the gene activity data  $Y_i$  has a multivariate<sup>29</sup>  
 30 normal distribution, i.e.,  $Y \sim \mathbb{P}_{\mu, \Sigma}$ , with mean  $\mu$  and covariance matrix  $\Sigma$ . Together<sup>30</sup>  
 31 with conditional independence graph  $G = (V, E)$ ,  $(G, \mathbb{P}_{\mu, \Sigma})$  constitutes a Gaussian<sup>31</sup>  
 32 graphical model or a covariance selection model [14]. This Gaussian graphical model<sup>32</sup>  
 33 puts some conditions on the covariance matrix  $\Sigma$ . Let  $\Theta = \Sigma^{-1}$  be the precision<sup>33</sup>

<sup>1</sup>or concentration matrix, then  $\Theta$  contains all conditional independence information <sup>1</sup>

<sup>2</sup>for the Gaussian graphical model. In particular, <sup>2</sup>

$$\theta_{ij} = 0 \iff (i, j) \notin E \iff Y_i \perp Y_j | Y_{-\{i,j\}}. \quad \begin{matrix} 3 \\ 4 \end{matrix}$$

<sup>5</sup>In fact, it is easy to show that given the set of  $E^c = \{(i, j) \mid \theta_{ij} = 0\}$ , a multivariate <sup>5</sup>  
<sup>6</sup>normal probability distribution  $f(\mathbf{y})$  can be factorized as a product of functions  $f$  <sup>6</sup>  
<sup>7</sup>which do not jointly depend to  $y_i$  and  $y_j$  when  $(i, j) \in E^c$ . <sup>7</sup>  
<sup>8</sup>

<sup>9</sup>Given a set of  $n$  observations on the Gaussian graphical model,  $y_1, \dots, y_n$ , the <sup>9</sup>  
<sup>10</sup>log-likelihood can be written as <sup>10</sup>

$$l(\mu, \Theta) = \frac{n}{2} \{ \log |\Theta| - \text{Tr}(S\Theta) - (\mu - \bar{y})^t \Theta (\mu - \bar{y}) \}, \quad \begin{matrix} 11 \\ 12 \end{matrix}$$

<sup>13</sup>where  $S = \sum_k (y_k - \bar{y})(y_k - \bar{y})^t / n$  is the sample covariance matrix. From the form <sup>13</sup>  
<sup>14</sup>of the likelihood, it is clear that  $\hat{\mu} = \bar{y}$  is the maximum likelihood estimate of  $\mu$  <sup>14</sup>  
<sup>15</sup>irrespective of the number of observations and the underlying graph  $G$ . For the <sup>15</sup>  
<sup>16</sup>MLE for  $\Theta$ , the story is more complicated. For a the complete graph, the maximum <sup>16</sup>  
<sup>17</sup>likelihood estimate is not uniquely defined when the number of observations is less <sup>17</sup>  
<sup>18</sup>than the number of vertices,  $n < |V|$ . This situation is really common for experi- <sup>18</sup>  
<sup>19</sup>ments to infer genomic networks. On the other hand, a gene-regulatory network is <sup>19</sup>  
<sup>20</sup>typically sparse, which means that the number of links is small with respect to the <sup>20</sup>  
<sup>21</sup>possible number of connections. This may mean that  $\Theta$  is estimable with respect <sup>21</sup>  
<sup>22</sup>to the underlying true sparse graph,  $G$ . The only problem is that we don't know <sup>22</sup>  
<sup>23</sup>which sparse graph that is. Therefore, we impose an additional constraint, <sup>23</sup>

$$\hat{\Theta}_\rho = \arg \max_{\Theta} l(\bar{y}, \Theta), \quad \begin{matrix} 24 \\ 25 \end{matrix}$$

<sup>26</sup>subject to <sup>26</sup>

$$\|\Theta\|_1 := \sum_{i=1}^{p-1} \sum_{j=i+1}^p |\theta_{ij}| < \rho, \quad \begin{matrix} 27 \\ 28 \end{matrix}$$

<sup>29</sup>where typically we do not penalize the diagonal of the precision matrix. Sparsity <sup>29</sup>  
<sup>30</sup>of the genomic network is not only our current best knowledge of system, but <sup>30</sup>  
<sup>31</sup>coincidentally it is also computational useful. Informally, a graph with few edges is <sup>31</sup>  
<sup>32</sup>sparse, whereas a graph with many edges is dense. [26] formally defines a graph <sup>32</sup>  
<sup>33</sup> $G = (V, E)$  to be sparse, if  $|E| = O(|V|)$ , where  $|V|$  is the number of vertices <sup>33</sup>

<sup>1</sup>and  $|E|$  is the number of edges. A graph  $G$  is said to be dense, if  $|E| = O(|V|^2)$ .<sup>1</sup>

<sup>2</sup>By constraining the estimate to satisfy an  $\ell_1$  constraint, it is possible to combine<sup>2</sup>

<sup>3</sup>estimation of the precision matrix  $\Theta$  with the estimation of the underlying graph<sup>3</sup>

<sup>4</sup> $G$ .<sup>4</sup>

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<sup>11</sup>3.2 Dynamic Gaussian graphical models<sup>11</sup>

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<sup>13</sup>In this section, we introduce the concept of a dynamic Gaussian graphical model,<sup>13</sup>

<sup>14</sup>which extends the static Gaussian graphical model that was introduced in the<sup>14</sup>

<sup>15</sup>previous section. We first define a dynamic graph  $G = (V, E)$ . Consider a set of<sup>15</sup>

<sup>16</sup>genes  $\Gamma = \{\gamma_1, \dots, \gamma_p\}$  and a set of time points where these genes were observed<sup>16</sup>

<sup>17</sup> $\tau = \{t_1, \dots, t_T\}$ . We define the vertices of the dynamic graph as the Cartesian prod-<sup>17</sup>

<sup>18</sup>uct of the genes and time points,  $V = \Gamma \times \tau$ . Therefore, a vertex in this graph is<sup>18</sup>

<sup>19</sup>an element  $(\gamma_g, t_s)$ . The edges are some subset of the Cartesian product of vertices,<sup>19</sup>

<sup>20</sup> $E \subset V \times V$ . An element of  $E$  will be written as  $\{(\gamma_g, t_s), (\gamma_{g'}, t_{s'})\}$ , stressing the fact<sup>20</sup>

<sup>21</sup>that it links one gene at a particular time point with another gene at another time<sup>21</sup>

<sup>22</sup>point. We will associate with each node of the graph a random variable  $Y_{gs}$ , which<sup>22</sup>

<sup>23</sup>represents the amount of gene activity of gene  $g$  at time  $s$ .<sup>23</sup>

<sup>24</sup><sup>24</sup>

<sup>25</sup> With the above ingredients, we can now define a **dynamic Gaussian graphical**<sup>25</sup>

<sup>26</sup>**model** as the tuple  $(G, \mathbb{P}_{\mu, \Theta})$ , where  $G = (\Gamma \times \tau, E)$  is a dynamic graph and  $\mathbb{P}_{\mu, \Sigma}$  is<sup>26</sup>

<sup>27</sup>a collection of multivariate Gaussian distributions with mean  $\mu$  and inverse covari-<sup>27</sup>

<sup>28</sup>ance matrix  $\Theta$ , that are compatible with the conditional independence relationships<sup>28</sup>

<sup>29</sup>described in the edge set  $E$ .<sup>29</sup>

<sup>30</sup><sup>30</sup>

<sup>31</sup> In principle, the ordering of the vertices is arbitrary. For interpretation purposes,<sup>31</sup>

<sup>32</sup>it helps to sort the vertices by time points and within time points by genes. This<sup>32</sup>

<sup>33</sup>results in a natural partition  $\{(N_l, S_l) \mid l = 0, \dots, t - 1\}$  of the inverse covariance<sup>33</sup>



matrix  $\Theta$ ,

$$\Theta = \begin{bmatrix} S_0^1 & N_0^1 & S_1^1 & N_1^1 & S_2^1 & N_2^1 & \dots & \dots \\ & S_0^1 & N_1^1 & S_1^1 & N_2^1 & S_2^1 & \dots & \dots \\ & & S_0^2 & N_0^2 & S_1^2 & N_1^2 & S_2^2 & N_2^2 \\ & & & S_0^2 & N_1^2 & S_1^2 & N_2^2 & S_2^2 \\ & & & & S_0^3 & N_0^3 & S_1^3 & N_1^3 \\ & & & & & S_0^3 & N_1^3 & S_1^3 \\ & & & & & & \ddots & \vdots \\ & & & & & & & \ddots \end{bmatrix},$$

where  $S_l$  represent the self-self interactions of the genes and  $N_l$  the network interaction between the genes, both at time lag  $l$ . The self-self interactions therefore represent the diagonal and subsequent off-diagonals of the matrix  $\Theta$ , whereas  $N_l$  are the diagonal blocks and subsequent off-diagonal blocks minus the diagonal. Each of this subsets can be further partitioned, as indicated by  $S_l^t$  and  $N_l^t$ . In these subpartitions,  $S_l^t$  is the self-self interaction of the genes at lag  $l$  and time  $t$ . Similarly,  $N_l^t$  are the network interactions at lag  $l$  and time  $t$ .

As the full dynamic Gaussian graphical model is still heavily parameterized with a typically big  $pT \times pT$  inverse covariance matrix, it makes sense to consider relevant subclasses. It is for example not particularly likely that two genes are related across a lot of time points, conditional on the intermediate states. We therefore define the **autoregressive Gaussian graphical model of order  $k$**  ( $G, \mathbb{P}_{\mu, \Theta}, AR(k)$ ) as a dynamic Gaussian graphical model ( $G, \mathbb{P}_{\mu, \Theta}$ ), for which

$$\forall l > k : N_l = S_l = 0.$$

This model assumes that genes are conditionally uncorrelated for time lags larger than  $k$ . In practice, we typically consider  $k = 1$  or  $k = 2$ , which from an interpretational point of view are most interesting. It is important to note that the autoregressive Gaussian graphical model is directly associated to a particular network structure  $G$ , which represents the conditional dependence graph of the random variables associated with the vertices of the graph.

### 13.3 Slowly changing dynamic graphical models

The main question this paper wants to answer is how to infer a meaningful biological dynamic network from noisy data on the nodes, such as, e.g., RNA seq values or protein levels. The two features we will assume particularly relevant of a gene network are its *sparsity* and its *persistence*. DNA, RNA and proteins are very specific molecules that are capable of interacting, typically, with only a very limited number of other molecules. This means that a genetic network is highly structured and selective, and, therefore, characterized by a high degree of sparsity. As genetic interactions depend very much on the basic molecular structure of its constitutive parts, the potential to interact between various genes will typically not change over time, unless particular regime changes in the cell affect its thermo-dynamic properties. Interactions in the dynamic network  $G$  therefore tend to persist over time. We will show in this subsection how we can incorporate these two ideas, sparsity and persistence of the network, in the inferential objective function by means of a penalized likelihood function.

In the T-cell experiment, we assume we have 44 observations from the  $57 \times 10^6$  dimensional autoregressive Gaussian graphical model of order  $k = 1$ . Not only do we want to infer a sparse network  $G$ , but also one for which the underlying network partitions  $N_l = \{N_l^1, \dots, N_l^t, \dots, N_l^T\}$  ( $l = 0, 1$ ) change only slowly across time  $t$ . This requires an additional set of constraints in our maximum likelihood inference. In general, we assume we have  $n$  observations  $y_1, \dots, y_n$ , each coming from the autoregressive  $k$  dynamic Gaussian graphical model  $(G, P_{\mu, \Theta}, AR(k))$ .

Given two tuning parameters  $\rho_1$  and  $\rho_2$ , we define a slowly changing dynamic network as the solution of the penalized maximum likelihood of the autoregressive  $k$  dynamic Gaussian graphical model,

$$l(\mu, \Theta) = \frac{n}{2} \{ \log |\Theta| - \text{Tr}(S\Theta) - (\mu - \bar{y})^t \Theta (\mu - \bar{y}) \}, \quad (1)$$

subject to

$$\|\Theta\|_1 < \rho_1 \quad (2)$$

$$\sum_{l=0}^k \sum_{s=1}^{T-1} \|N_l^s - N_l^{s+1}\|_1 < \rho_2 \quad (3)$$

$$\forall l > k : N_l = S_l = 0 \quad (4)$$

<sup>1</sup>Whereas the first constraint induces a generally sparse dynamic network, the second<sup>1</sup>  
<sup>2</sup>constraint penalizes large changes in the network coefficients, thereby inducing a<sup>2</sup>  
<sup>3</sup>slowly changing or persistent network through time. Therefore, the penalty param-<sup>3</sup>  
<sup>4</sup>eters are directly related to the zero structure of the estimate  $\hat{\Theta}_{\rho_1, \rho_2}$  and, therefore,<sup>4</sup>  
<sup>5</sup>to the estimate of the dynamic genetic graph  $\hat{G}_{\rho_1, \rho_2}$ .<sup>5</sup>

<sup>6</sup> Solving the above penalized maximization problem is an active field of research<sup>6</sup>  
<sup>7</sup>in the optimization field. We use the log determinant proximal point approximation<sup>7</sup>  
<sup>8</sup>method developed by [23]. Each constraint gets coded into a linear map. We consider<sup>8</sup>  
<sup>9</sup> $A(\Theta) = \|\Theta\|_1$  associated with constraint (2),  $B(\Theta) = \sum_{l=0}^k \sum_{s=1}^{T-1} \|N_l^s - N_l^{s+1}\|_1$ <sup>9</sup>  
<sup>10</sup>associated with constraint (3) and  $C_l(\Theta) = (S_l; N_l)$  associated with constraint (4).<sup>10</sup>

<sup>11</sup>This method introduces two sets of slack variables to deal with the two inequality<sup>11</sup>  
<sup>12</sup>constraints. The constraint optimization problem (1) is now written as:<sup>12</sup>

$$\hat{\Theta} := \underset{\Theta}{\operatorname{argmin}} \{-\log |\Theta| + \operatorname{Tr}(\mathbf{S}\Theta) + \lambda_1 \mathbf{v}^+ + \lambda_1 \mathbf{v}^- + \lambda_2 \mathbf{w}^+ + \lambda_2 \mathbf{w}^-\}$$

$$\begin{aligned} \text{subject to} \quad & \mathbf{A}(\Theta) - \mathbf{v}^+ + \mathbf{v}^- = \mathbf{0} \\ & \mathbf{B}(\Theta) - \mathbf{w}^+ + \mathbf{w}^- = \mathbf{0} \\ & \mathbf{C}_l(\Theta) = \mathbf{0}, \quad l = 0, \dots, k \\ & \Theta \succ 0, \mathbf{v}^+, \mathbf{v}^-, \mathbf{w}^+, \mathbf{w}^- \geq \mathbf{0}, \end{aligned}$$

where  $\lambda_1$  and  $\lambda_2$  are functions of  $\rho_1$  and  $\rho_2$  respectively. In this format, the opti-  
mization can be solved directly by LogDetPPA.

The non-negative tuning parameters  $\lambda_1$  and  $\lambda_2$  effectively determine the sparsity  
and the stability through time of the network, respectively. Selecting these tuning  
parameters is a form of model selection. Depending on the interests of the user,  
which can be maximizing posterior model probability or minimizing prediction er-  
ror, either a BIC-type criterion or an AIC-type criterion is proposed. We consider a  
grid of values  $(\lambda_1, \lambda_2)$  and minimize information criterion scores such as AIC, AICc,  
and BIC. Then we use stability selection to select a more stable graph [27].

*Example: T-cell* We consider a subset of the T-cell data to illustrate the per-  
formance of the autoregressive Gaussian graphical model approach with a slowly  
changing network penalty. Only 4 genes and 2 time points were considered. Table 1

<sup>1</sup>shows the estimated precision matrix, fixing the tuning parameters  $\lambda_1 = 0.01$  and<sup>1</sup>  
<sup>2</sup> $\lambda_2 = 0.1$ . It can be seen that  $N_0^1$  is a network with three edges  $(1, 3), (1, 4), (2, 4)$ ,<sup>2</sup>  
<sup>3</sup>which in the next time point  $N_0^2$  slowly changes to another network with edges<sup>3</sup>  
<sup>4</sup> $(1, 2), (1, 4), (2, 4)$ . In section 4.3 we consider the full dataset. 4

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## <sup>6</sup>4 Results 6

### <sup>7</sup>4.1 Comparison with other methods 7

<sup>8</sup>In this section we compare the dynamic network inference method with other meth-<sup>8</sup>  
<sup>9</sup>ods proposed in the literature to estimate networks. [28] suggest a procedure based<sup>9</sup>  
<sup>10</sup>on large-scale hypothesis testing of partial correlations in combination with false<sup>10</sup>  
<sup>11</sup>discovery rate cut-offs, implemented in the R-package **GeneNet**. [29] propose an em-<sup>11</sup>  
<sup>12</sup>pirical bayes method for estimating biological networks from temporal microarray<sup>12</sup>  
<sup>13</sup>data. Their method aims to infer a directed graphical model, a so-called Bayesian<sup>13</sup>  
<sup>14</sup>network, that remains constant through time. This method is implemented in the<sup>14</sup>  
<sup>15</sup>R-package **ebdbNet**. There is a whole class of methods based on the graphical lasso<sup>15</sup>  
<sup>16</sup>[20]. Besides the original method, [22] proposed a factorial graphical lasso, imple-<sup>16</sup>  
<sup>17</sup>mented in the **sglasso** R-package and [30] consider a sparse autoregressive network<sup>17</sup>  
<sup>18</sup>inference method using undirected graphical models, implemented in the R-package<sup>18</sup>  
<sup>19</sup>**SparseTSCGM**. 19

<sup>20</sup>We simulate data from a network along six time-points that is affected by a 20  
<sup>21</sup>regime change between time points 3 and 4. Figure 1 shows the original networks,<sup>21</sup>  
<sup>22</sup>interpreted as lag zero conditional independence graphs  $N_0$ . We simulate  $n = 100$ <sup>22</sup>  
<sup>23</sup>observations and report the results of the methods described above. Due to the 23  
<sup>24</sup>large number of links, GeneNet by default corrects for multiple testing, resulting in 24  
<sup>25</sup>a very sparse graph. In fact, it merely detects a seven edges throughout the whole 25  
<sup>26</sup>time-course, when correcting at the 0.9 local fdr rate. In Figure 2, we lowered the 26  
<sup>27</sup>local fdr to 0.5, which allows us to pick up additional edges, but clearly it lack 27  
<sup>28</sup>consistency across the various time points. Roughly the same results crystalize,<sup>28</sup>  
<sup>29</sup>when applying separate graphical lassos to each of the time-points. The tuning 29  
<sup>30</sup>parameter is selected by using the RIC. Figure 3 shows that some structure of the 30  
<sup>31</sup>underlying graph has been recovered, but with disappointing consistency across 31  
<sup>32</sup>the time-points. Factorial graphical lasso, sparse TSCGM and ebdbNet all infer a 32  
<sup>33</sup>constant graph across time, which indeed captures some aspects of the underlying 33

<sup>1</sup>structure, but fails to detect the change point (cf. Figure 4). Although not perfect,<sup>1</sup>  
<sup>2</sup>the slowly changing graphical model approach correctly borrows strength across the<sup>2</sup>  
<sup>3</sup>6 time-points to more accurately infer the underlying graph and at the same time<sup>3</sup>  
<sup>4</sup>to correctly detect the underlying changes in the dynamics (cf. Figure 5).<sup>4</sup>

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5

#### <sup>6</sup>4.2 Simulation study<sup>6</sup>

<sup>7</sup>We perform a simulation study to show the performance of the autoregressive Gaus-<sup>7</sup>  
<sup>8</sup>sian graphical model of order one. We consider four different scenarios with a varying<sup>8</sup>  
<sup>9</sup>number of genes  $p \in \{20, 40, 60, 80\}$ , each with  $n = 50$  observations across  $T = 3$ <sup>9</sup>  
<sup>10</sup>time points. For each scenario we simulate 100 datasets from a multivariate normal<sup>10</sup>  
<sup>11</sup>distribution with  $\boldsymbol{\mu}$  equal to zero and  $\boldsymbol{\Sigma}$  equal to the inverse of a precision matrix<sup>11</sup>  
<sup>12</sup> $\boldsymbol{\Theta}$ . The structure of the graph slowly changes across time and observations are con-<sup>12</sup>  
<sup>13</sup>ditionally independent for time lags greater than one. Note that in all four scenarios<sup>13</sup>  
<sup>14</sup>the number of replicates  $n$  is fewer than the number of random variables  $pT$ .<sup>14</sup>

<sup>15</sup>Table 2 shows the average of false positive, false negative, false discovery, false<sup>15</sup>  
<sup>16</sup>non-discovery rates as well as the average  $F_1$  score over 100 simulations. We use<sup>16</sup>  
<sup>17</sup>the corrected and normal AIC, as well as the BIC to select the tuning parameters in<sup>17</sup>  
<sup>18</sup>the models. The corrected AIC adds an additional penalty to account for the small<sup>18</sup>  
<sup>19</sup>number of observations. These results show that the slowly changing autoregressive<sup>19</sup>  
<sup>20</sup>Gaussian graphical model is very reliable even with small numbers of observations<sup>20</sup>  
<sup>21</sup>and that it can be used for real applications when few changes in different time<sup>21</sup>  
<sup>22</sup>points are present using any type of model selection method.<sup>22</sup>

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#### <sup>23</sup>4.3 Application to T-cell<sup>23</sup>

<sup>24</sup>In this subsection we apply an autoregressive Gaussian graphical model of order one<sup>24</sup>  
<sup>25</sup>to the human T-cell dataset. We assume that genes which are two time points apart,<sup>25</sup>  
<sup>26</sup>i.e.  $Y_{s,t}$  and  $Y_{s,t+2}$ , are conditional independent given the intervening observations.<sup>26</sup>  
<sup>27</sup>This means that the edge set for networks at lag 2, i.e.  $N_2$ , is an empty set. Figure<sup>27</sup>  
<sup>28</sup>6 is obtained from the estimation procedure. The two upper graphs show the two<sup>28</sup>  
<sup>29</sup>networks at time points 1 and 2, respectively. The bottom left figure, “Intersection,”<sup>29</sup>  
<sup>30</sup>shows the large overlap between the two networks, induced by the significant tuning<sup>30</sup>  
<sup>31</sup>parameter  $\rho_2$ . On the other hand, the bottom right figure shows the changes between<sup>31</sup>  
<sup>32</sup>these two time points. It shows, for example, that initially MCL1, a pro-survival<sup>32</sup>  
<sup>33</sup>BCL2 family member, is a highly connected node in the T-cell network. It is known<sup>33</sup>

<sup>1</sup>that SCF(FBW7) regulates cellular apoptosis by targeting MCL1 for ubiquitylation<sup>1</sup>  
<sup>2</sup>and destruction [31]. This is probably why initially MCL1 loses connections to other<sup>2</sup>  
<sup>3</sup>genes. 3

<sup>4</sup> 4

## <sup>5</sup> **5 Conclusion** 5

<sup>6</sup> Many time-course genomic experiments are performed in order to discover certain 6  
<sup>7</sup> regime changes that may be taking place during that period. Under these circum- 7  
<sup>8</sup> stances, representing genomic interactions by means of a static graphs may be 8  
<sup>9</sup> misleading. Certainly, it would fail to detect any changes in the topology of the 9  
<sup>10</sup> network. We use a sparse dynamic graphical model to infer the underlying slowly 10  
<sup>11</sup> changing network. One of the major contributions is that this methodology is capa- 11  
<sup>12</sup> ble of providing fast inference about the dynamic network structure in moderately 12  
<sup>13</sup> large networks. Until now, even sparse static inference could be painstakingly slow 13  
<sup>14</sup> and would typically lack obvious interpretation. We applied the method to a human 14  
<sup>15</sup> t-cell dataset to study the developmental aspects of the sparse genomic interactions. 15  
<sup>16</sup> One result, backed up by recent research, is that MCL1 is targeted early on and 16  
<sup>17</sup> thereby loses its connections to the rest of the genomic network. 17

<sup>18</sup> 18  
<sup>19</sup> Once a graph has been estimated and changes have been evaluated, other ques- 19  
<sup>20</sup> tions on how to analyze time-evolution networks might be posed. Does the network 20  
<sup>21</sup> retain certain graph properties as it grows and evolves? Does the graph undergo 21  
<sup>22</sup> a phase transition, in which its behaviour suddenly changes? In answering these 22  
<sup>23</sup> questions it is of interest to have a diagnostic tool for tracking graph properties 23  
<sup>24</sup> and noting anomalies and graph characteristics of interest. For example, a useful 24  
<sup>25</sup> tool is ADAGE [32], which is a software package that analyzes the number of edges 25  
<sup>26</sup> over time, the number of nodes over time, the densification law, the eigenvalues 26  
<sup>27</sup> over increasing nodes, the size of the largest connected component, the number 27  
<sup>28</sup> of connected components versus nodes and time and the comparative sizes of the 28  
<sup>29</sup> connected components over time. 29

<sup>30</sup> 30

### <sup>31</sup> **Competing interests** 31

<sup>32</sup> The authors declare that they have no competing interests. 32

### <sup>33</sup> **Author's contributions** 33

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## 12 Figures 12

13 **Figure 1 A regime change between time-points 3 and 4.** Data are simulated from a network 13  
14 that is subject to a regime change between time-points 3 and 4. 14

15 15

16 **Figure 2 GeneNet's performance.** GeneNet infers links by means of multiple testing. By lowering 16  
17 the local *fdr* to 0.5, we recover some of the network structure, but consistency across the 17  
18 time-points is absent. 18

19 19

20 **Figure 3 Graphical lasso's performance.** Graphical lasso estimates a sparse network for each of 20  
21 the time points separately. Although it recovers some of the network structure, there is little 21  
22 consistency across the time-points. 22

23 23

24 **Figure 4 Performance of Facorial Graphical lasso, Sparse TSCGM and ebdbNet.** Facorial 24  
25 Graphical lasso, Sparse TSCGM and ebdbNet each recover a constant network across the 6 time 25  
26 points. It shows the general underlying structure of the network, but fails to detect the change 26  
27 point. 27

28 28

29 **Figure 5 Performance of the Slowly Changing Graphical model.** The slowly changing graphical 28  
30 model misses some of the timings of the changes, but correctly identifies the regime change and it 29  
31 recovers the underlying structure of the changing network. 30

31 31

32 **Figure 6 T-cell network change between  $t = 1$  and  $t = 2$ .** The lag zero network  $N_0$  for the 32  
33 T-cell data with changes between time points  $t = 1$  and  $t = 2$ . 33



1 Tables

2 **Table 1** Conditional covariance  $\hat{\Theta}$  based on 44 replicates for 4 genes measured across 2 time points.  
 3 The tuning parameters  $\lambda_1$  and  $\lambda_2$  were fixed to 0.01 and 0.1, respectively.

Time	Gene	1				2			
		ZNF	CCN	SIV	SCY	ZNF	CCN	SIV	SCY
1	ZNF	1.24	0.00	-0.26	0.18	-0.22	-0.11	-0.11	-0.07
	CCN	-	1.49	0.00	-0.17	-0.18	-0.84	0.06	0.12
	SIV	-	-	1.44	0.00	-0.15	0.08	-0.69	-0.01
	SCY	-	-	-	1.19	0.02	0.13	0.41	-0.10
2	ZNF	-	-	-	-	1.07	-0.02	0.00	0.12
	CCN	-	-	-	-	-	1.55	0.00	0.24
	SIV	-	-	-	-	-	-	1.52	0.00
	SCY	-	-	-	-	-	-	-	1.08

11 **Table 2** The average of performance of various model selection algorithms for the four simulation  
 12 scenarios using four model selection methods in term of the fraction of correctly estimated  
 13 link/non-links, i.e. false positives (FP), false negatives (FN), false discoveries (FD) and false  
 14 non-discoveries (FnD), as well as the  $F_1 = (2 - 2FN)/(2 - FN + FP)$  score, which measures the  
 overall average accuracy of recall and precision. The best scores are indicated by bold font.

p		$\overline{FP}$	$\overline{FN}$	$\overline{FD}$	$\overline{FnD}$	$F_1$ score
20	AICc	<b>0.0092</b>	0.0811	<b>0.2000</b>	0.0031	0.9532
	BIC	0.0363	0.0139	0.4873	0.0005	<b>0.9751</b>
	AIC	0.0698	<b>0.0069</b>	0.6470	<b>0.0003</b>	0.9628
40	AICc	<b>0.0057</b>	0.0447	<b>0.2899</b>	0.0006	0.9743
	BIC	0.0088	0.0321	0.3826	0.0005	<b>0.9793</b>
	AIC	0.0437	<b>0.0041</b>	0.7514	<b>0.0001</b>	0.9766
60	AICc	<b>0.0016</b>	0.4585	<b>0.2730</b>	0.0036	0.7018
	BIC	<b>0.0016</b>	0.4585	<b>0.2730</b>	0.0036	0.7018
	AIC	0.0288	<b>0.1452</b>	0.8088	<b>0.0012</b>	<b>0.9076</b>
80	AICc	<b>0.0091</b>	0.1034	<b>0.1680</b>	0.0052	0.9410
	BIC	0.0396	0.0517	0.4527	0.0027	0.9541
	AIC	0.0670	<b>0.0000</b>	0.5704	<b>0.0000</b>	<b>9675</b>