

# Optimal Inference of Asynchronous Boolean Networks

Guy Karlebach<sup>1</sup>

<sup>1</sup>Department of Computer Science, Fitchburg State University, Pearl Street, Fitchburg, 01420, MA, USA.

Contributing authors: [gkarleba@fitchburgstate.edu](mailto:gkarleba@fitchburgstate.edu);

## Abstract

The network inference problem arises in biological research when one needs to quantitatively choose the best protein-interaction model for explaining a phenotype. The diverse nature of the data and nonlinear dynamics pose significant challenges in the search for the best methodology. In addition to balancing fit and model size, computational efficiency must be considered. Importantly, underlying the measurements, which are affected by experimental noise, there is a complex computational mechanism that is inherently hard to identify. To address these difficulties, we present a novel approach that uses algorithmic complexity to infer a Boolean network model from experimental data. We present an algorithm that is optimal within this framework and allows for asynchronous network dynamics. Furthermore, we show that using our methodology a solution to the pseudo-time inference problem, which is pertinent to the analysis of single-cell data, can be intertwined with network inference. Results are described for real and simulated datasets.

**Keywords:** Boolean Network, Inference, Optimization, Time-series, Gene Regulatory Network

## 1 Introduction

Research in molecular biology often aims to reveal a mechanistic understanding of underlying processes, in contrast to correlations between observations. In particular, one aims to describe cellular networks of interactions between proteins and other molecules that generate a specific phenotype [1–4]. Often, these networks display complex, nonlinear dynamics due to combinatorial interactions between regulators and

feedback loops in the topology. [5]. A key aspect of the problem is the Curse of Dimensionality, which ensures that the number of networks and datasets grows exponentially with the number of involved proteins, making inference susceptible to overfitting. Gene regulatory networks, which control the expression of genes in the cell, are an important type of biological networks that are studied using transcriptomic technologies. These technologies measure the level (expression) of all the genes in a sample and, more recently, in single cells [6], allowing the detection of regulatory relationships between them. When the measurement is at the sample level, we refer to the data as RNA-Sequencing data (RNA-Seq), and when it is at the single-cell level as single-cell RNA-Seq (scRNA-Seq). Numerous, fundamentally different inference methodologies have been proposed. For example, Keyl et al. used an explainable AI approach and layer-wise relevance propagation in order to select gene regulators that are ranked as important predictors in a neural network model [7]. SCODE is a method for inferring ordinary differential equations from scRNA-Seq data, such that the regulatory relationships can be derived from the equations [8]. CEFCON uses a graph neural network with an attention mechanism for constructing the regulatory network from prior network knowledge, scRNA-Seq data and trajectory information [9]. Chen et al. used a genetic algorithm to reconstruct a Probabilistic Boolean Network from scRNA-Seq data, a model that accommodates probabilistic transitions in a Boolean network model [10]. However, none of the existing methodologies satisfy all of the following criteria: define an objective criterion for comparing any pair of networks, find optimal solution with respect to this inference criterion, regularize any solution as part of the inference criterion and process both steady-state and time-series data. Presenting such a methodology is the goal of this paper.

We will show that using a computational definition of randomness leads to an inference methodology that satisfies the requirements described above, using Boolean networks as the modeling language [11–13].

Another challenge that arises specifically when working with scRNA-Seq is that individual measurements originate from a time-series but a temporal order is not provided with the dataset. In other words, we obtain "snapshots" of the cell at different states, that may have occurred consecutively in time and thus describe a network trajectory, but we do not know at which point in the trajectory each state, or snapshot, occurred. Here, too, many efforts have been devoted to the development of inference methods such that the cell order, known as pseudo-time, could be derived. To name a few examples, Riba et al. developed DeepCycle, a method that predicts position relative to the cell cycle based on the relationship between spliced and unspliced transcripts. DeepCycle trains an autoencoder that encodes the relationship between spliced and unspliced scRNA-Seq reads as the transcriptional phase of the cell, a latent variable that maps a cell into a position on the periodic cell cycle trajectory [14]. Liu et al. introduced reCAT, a method for cell-cycle stage inference that finds a cycle that minimizes transcriptional differences between adjacent cells [15]. The CONFESS method utilizes image information to reconstruct single-cell dynamics [16]. PAGA creates a map connecting cell groups based on the estimation of the connectivity of manifold partitions [17]. GraphDDP combines clusters into differentiation trajectories using a forced-based graph layout approach [18]. We argue that the pseudo-time inference

problem is closely related to the network inference problem and that a network model should be utilized to solve it. We will demonstrate how this can be done in the context of the novel methodology suggested here.

## 2 Methods

### 2.1 Network Inference

Discussing the inference of a Boolean network model from gene expression data requires some basic terminology. A *state* (of a biological cell) corresponds to a multidimensional vector, where each vector entry is the level of one gene. This level describes the degree to which a gene is active, and for our purposes it can take only two values - 0 (inactive) and 1 (active). A Boolean network can be viewed as a function that maps a Boolean state to another, potentially identical, state. For a given network, a sequence of states such that each state maps to its successive state in the sequence is called a *trajectory*. A trajectory whose states are repeated indefinitely by the network is known as an *attractor*. An attractor consisting of a single state is referred to as a *steady state*. By definition, steady states and trajectories correspond to a specific model. To provide some biological context, changes in gene expression during cellular differentiation will result in a trajectory that can be modeled using a gene regulatory network, described as a Boolean network. The trajectories and steady states generated by a network are known as the network *dynamics*. A dataset is a collection of measurements of trajectories and steady states. These measurements can be affected by noise, in which case states do not perfectly match the mapping defined by the network because some gene levels are incorrectly measured (*noise bits*). The goal of the inference problem is to find the network that generated the dataset. It should be clarified here that there are no hidden variables in the problem - the value of every gene is always observed. This is a property of the experimental techniques that generate the data. While adding unobserved variables does not overly complicate the methodology, it is not required for our purposes. A network can be described concisely by a directed graph  $G(V,E)$ , where  $G$  is a set of nodes or genes, and  $E$  are edges such that a gene  $g$ 's regulators are the nodes from which directed edges extend to  $g$ , i.e.  $u : (u, g) \in E$ . Each set of  $n$  regulators of a gene is associated with a logic function that has  $n$  Boolean inputs and one Boolean output. The Boolean inputs are determined when the nodes that are associated with the function's inputs are assigned Boolean values. Biologically, the input nodes correspond to regulators, and their combined activity or inactivity determines the Boolean value of their target gene. Given a Boolean assignment to all the nodes in the network, its state is defined, and the next state can be computed by combining the outputs of all the logic functions. If the change in gene levels can be deferred, such that it is not immediately applied to the consecutive state, we say that the network is asynchronous. In that case, the function defined by the network as a whole is non-deterministic. Otherwise, the network is said to be synchronous.

A gene expression dataset as a  $N \times M$  matrix, where  $N$  corresponds to the number of genes whose expression level was measured, and  $M$  corresponds to the number of experiments. The entry at indices  $i,j$  contains a Boolean value that is equal to 1 if the gene is in the active state, and otherwise equal to 0. Initially, we assume that the order

between states is known for every trajectory. This is the case for biological datasets in which the cells have been synchronized or in which the cells are at steady state. An algorithm for inferring pseudo-time follows from the basic methodology, and will be detailed later on. We will also assume that a set of plausible regulatory interactions has been determined for each gene. This can be achieved using other experimental technologies like ChIP-Seq [19], or by keeping regulators whose levels correlate with their target's. From this initial set, the modeler wants to choose the optimal subset, including the logic tables by which the regulators determine the state of the target. For mapping biological measurements to Boolean values we will use existing methods [20–23], and in this section we assume that all gene values are already Boolean.

The methodology that we present is based on the concept of Kolmogorov complexity [24]. Randomness is not defined using probabilities, but rather as datasets that cannot be generated by a Boolean network with a significantly shorter encoding. Since the number of Boolean networks with a significantly shorter encoding is exponentially smaller, only datasets that are not random will have a model that can generate them. This leads to an optimization criterion: find a network that can be encoded with the smallest number of bits, that matches as many bits as possible in the dataset, where all bits have the same weights.

One has to note that datasets may only contain any trajectory or steady state once. The question that we ask is whether these could be generated by a concise Boolean network, and thus multiple occurrences of the exact output do not alter the randomness or non-randomness of the dataset, just as running a computer program again and getting the same output does not change our conclusions about the nature of the program.

Another potential source of randomness can be the computation itself, i.e. when the network displays non-determinism in shifting between states. Every non-deterministic step branches between two possible states, and so intuitively doubles the number of states generated by the network. The number of such steps can then be added to the sum that we wish to minimize.

In order to find an optimal solution to the problem, we formulate it as 0/1 Integer programming. The variables of the problem are denoted by uppercase English letters. A  $B$  variable is defined for every measurement, i.e. an entry in the expression matrix that describes a gene and its activity at a given state, and is equal to 1 if there is a mismatch between the observed value of the gene at that measurement and the value that the model assigns it. An  $I$  variable is defined for every combination of regulator values, and is equal to 1 if the state of the target gene is set to 1 for that combination, and otherwise it is equal to 0. An  $R$  variable is defined for every (potential-regulator,target) pair. It is equal to 1 if the regulator is chosen in the optimal solution, and otherwise to 0. A  $V$  variable is defined for every gene and possible number of regulators for that gene, and is equal to 1 if the gene has at least that number of regulators, and otherwise it is equal to 0. Later in this section we will also define a  $D$  variable, which will allow us to implement asynchronous dynamics. Using these variables, we first describe the constraints of the model, and then the objective function: Let  $C_{i,j}$  denote the observed Boolean value of gene  $i$  at experiment  $j$ . The

corresponding  $B$  variable is  $B_{g_i,j}$ , and it is equal to 1 if the value of gene  $g_i$  in experiment  $j$  does not match the model's assignment, and otherwise 0. If  $j$  is the index of a steady state in the data,  $g_{k+1}$  is a gene with regulators  $g_1, g_2, \dots, g_k$ , we go over every possible combination of values for these regulators  $(w_1, w_2, \dots, w_k)$ ,  $w_j \in \{0, 1\}$  and for each combination add the following constraint:

$$\begin{aligned} & \sum_{r=1}^k (C_{r,j} \cdot (w_r + (1 - 2 \cdot w_r) \cdot B_{g_r,j})) \\ & + (1 - C_{r,j}) \cdot ((1 - w_r) + (2 \cdot w_r - 1) \cdot B_{g_r,j}) \\ & + C_{k+1,j} \cdot B_{g_{k+1},j} + (1 - C_{k+1,j}) \cdot (1 - B_{g_{k+1},j}) \\ & < (2 - I(w_1, w_2, \dots, w_k)) \cdot (k + 1) \end{aligned} \quad (1)$$

where  $I(w_1, w_2, \dots, w_k)$  is the output of the Boolean function that determines the value of  $g_{k+1}$ . This constraint means that if the output variable  $I(w_1, w_2, \dots, w_k)$  was set to 1, whenever the combination  $w_1, w_2, \dots, w_k$  appears, the output (the value of  $g_{k+1}$ ) must be 1. If the data contains trajectories, the observed values of the target gene and the corresponding 0/1 IP variables will be taken from the subsequent time point, at which the regulation is expected to take effect if the model is synchronous. Similarly, we add the following constraint to account for the case where  $I(w_1, w_2, \dots, w_k)$  is set to 0,:

$$\begin{aligned} & \sum_{r=1}^k (C_{r,j} \cdot (w_r + (1 - 2 \cdot w_r) \cdot B_{g_r,j})) \\ & + (1 - C_{r,j}) \cdot ((1 - w_r) + (2 \cdot w_r - 1) \cdot B_{g_r,j}) \\ & + C_{k+1,j} \cdot (1 - B_{g_{k+1},j}) + (1 - C_{k+1,j}) \cdot B_{g_{k+1},j} \\ & < (I(w_1, w_2, \dots, w_k) + 1) \cdot (k + 1) \end{aligned} \quad (2)$$

Next, for every gene  $g_i$  and each one of its regulators  $g_j$ , we create a Boolean variable  $R_{i,j}$ . In other words, every potential regulator of gene  $g_i$  is associated with an  $R$  variable for that gene. For every two different assignments of values to  $g_i$ 's regulators, i.e. inputs to the logic function that sets the value of  $g_i$ , the sum of  $R$  variables of regulators which have different values in the two assignments is constrained to be greater than the differences between the  $I$  variables that determine the outputs for these assignments. For example, with two regulators  $R_1$  and  $R_2$  and two assignments 01 and 00 to the variables, respectively,  $R_2$  must be greater than  $I_{01} - I_{00}$  and also greater than  $I_{00} - I_{01}$ . If the outputs for these two assignments are different, only the change in  $R_2$  can explain this difference, as  $R_1$  has the same value in both assignments. More generally, this constraint means that two different outputs can never occur for the exact combination of regulatory inputs, for otherwise the regulatory logic is not a function. The  $V_{ik}$  variable, which is defined for gene  $i$  and every possible number of regulators  $k$  of that gene, is constrained to be greater than the mean of the gene's  $R$  variables minus  $\frac{0.5}{\text{indegree}(g_i)}$  if  $k = 1$  or  $(\frac{i-1}{\text{indegree}(g_i)})$  if  $k > 1$ , where  $\text{indegree}(g_i)$  is the number of candidate regulators (or  $R$  variables) of  $g_i$ . To set the weights of variables in the objective to match the inference criterion, a weight of 1 is

given to  $B$  variables. Now if  $r$  regulators are chosen for a gene, all its  $V$  variables 1.. $r$  will be set to 1. Therefore, we set the weight of the first  $V$  variable of the gene to be the logarithm (base 2) of the number of ways to choose a first regulator plus the  $\log_2$  of the number of logic tables possible for one regulator. We then set the weight of the second  $V$  variable of the gene to be the  $\log_2$  of the number of ways to choose a second regulator after the first one was already chosen plus the  $\log_2$  of the number of logic tables with two regulators, minus the  $\log_2$  of the number of logic tables with one regulator. So the costs of encoding the logic tables cancel out by consecutive  $V$  variables, while the cost of choosing the regulators is produced by the combination of all the  $V$  that are set to 1. If we denote the number of logic tables with  $k$  regulators as  $L_k$ , the weight of the  $k^{\text{th}}$   $V$  variable is set to  $\log_2(L_k) - \log_2(L_{k-1}) + \log_2(\binom{N-k+1}{k})$

So far, we assumed that the updates of the model are synchronous. We now adapt the 0/1 IP formulation to fit asynchronous dynamics. We add a new type of variable called the  $D$  variable. This variable is defined for every constraint that involves the  $B$  variables in a trajectory, as defined in (1) and (2). It is added to the right hand side of the constraint, and therefore if it is equal to 1 it allows the output of the logic function to not agree with its inputs. We further constrain the  $D$  variable to be smaller than 1 minus the differences between the chosen value of target gene at the state at which the regulatory effect is taking effect and the previous state, i.e., the values selected for the gene by the model at these states. The latter constraint only allows the output of the logic function to disagree with its input if the output does not change, i.e. if the regulatory update is not immediate. Using the same notation as before, the additional constraints on the  $D$  variable can be described as follows:

$$1 - (C_{k+1,j+1} * (1 - B_{g_{k+1,j+1}}) + (1 - C_{k+1,j+1}) * B_{g_{k+1,j+1}} - (C_{k+1,j} * (1 - B_{g_{k+1,j}}) + (1 - C_{k+1,j}) * B_{g_{k+1,j}})) \geq D \quad (3)$$

$$1 - (C_{k+1,j} * (1 - B_{g_{k+1,j}}) + (1 - C_{k+1,j}) * B_{g_{k+1,j}} - (C_{k+1,j+1} * (1 - B_{g_{k+1,j+1}}) + (1 - C_{k+1,j+1}) * B_{g_{k+1,j+1}})) \geq D \quad (4)$$

Finally, for each target gene we constrain the first  $D$  variable in each trajectory to be smaller or equal to the sum of the target's  $R$  variables, such that the target would only be able to use the  $D$  variables if it has at least one regulator assigned to it.

We set the weight of every  $D$  variable to 1 in the objective function. Consider a network  $M$  that is the optimal solution for some dataset  $T$ . If it sets the value of the  $D$  variable to 1 at some time  $t$ , then the corresponding target must exert a regulatory change on one of its own targets after the delay introduced by the  $D$  variable, for otherwise a better solution could have been obtained without setting the  $D$  variable. Therefore, the trajectory of the model when there is no delay (i.e. when the  $D$  variable is not set) is different than the one it uses in the optimal solution. Now if we set the  $D$  variable to 0 instead, set the suffix of the trajectory from time  $t$  to fit exactly the trajectory of the model that was fit to  $T$  from time  $t$  and afterwards, then  $M$  must also be optimal for this new trajectory  $T'$ . If not, and there is another better fit model, then when flipping the bits back, it will still be better than  $M$  on the original dataset, whether  $M$  uses the  $D$  variable or not. Therefore, like the  $B$  variables, every  $D$  variable is equivalent

to one bit in the encoding of the network. The value of the objective function is a sum of the weights of variables that are set to 1 in the solution, i.e.:

$$\sum_{i=1, j=1}^{N, M} (B_{g_{i,j}} + W(V_{g_{i,j}}) \cdot V_{g_{i,j}} + D_{g_{i,j}}) \quad (5)$$

where  $W$  is a function that maps between a  $V$  variable and the weight assigned to it. This concludes the 0/1 Integer Programming formulation.

Powerful solvers like Gurobi [25] have dramatically improved our ability to solve 0/1 Integer Programming problems. Custom heuristics can be integrated with the solver to improve performance. We now describe such heuristics.

Perhaps the simplest heuristic for a trajectory is to perform a single pass over the data, state by state starting from the first state, and to record every input-output pair observed as long as it does not conflict with pairs observed before it. When a conflict occurs, the value of the target gene is flipped to match the output that was previously observed. A more sophisticated approach was suggested by Karlebach and Robinson [26], and can be applied to an expression data set composed of either steady states or equal-length trajectories:

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**Algorithm 1** Heuristic Search

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1. Choose a set of regulators.
  2. If the set has a single steady state, return it as a solution.
  3. If the set has a single trajectory, solve any inconsistencies using the single-pass heuristic, and return it as a solution.
  4. If the size of the set of steady states or trajectories is larger than 1 but the set is consistent with the regulators, return that set of states as a solution, possibly removing some redundant regulators by backward elimination.
  5. Otherwise, cluster the states and round the cluster centers into Boolean vectors, then solve the problem recursively for the cluster centers. The recursive call returns a set of consistent states  $S$ . For every state in the original set, choose its closest neighbor in  $S$ , and flip its values one by one to match the neighbor's values until all inconsistencies with states in  $S$  have been resolved, or until it is equal to the neighbor, which is already consistent. At that point add it to  $S$  so it can be compared to states that have not been made consistent yet. At the end of the process, return  $S$  excluding the cluster centers.
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The set of regulators in step 1 can be chosen from the current LP solution, for example all regulators which correspond to an  $R$  variable with value of at least 0.5. If the dataset contains both steady states and trajectories, then the recursive heuristic can be run for the steady states, and then the resulting logic can be used to remove inconsistencies from the trajectories using the single-pass heuristic. If trajectories have different lengths, equal-sized contiguous subsequences of trajectories can be solved by the recursive heuristic, and the remaining inconsistencies then resolved by

the single-pass heuristic. Care should be taken that clustering of these subsequences is biologically meaningful, for otherwise poor solutions may be result due to their incompatibility.

It remains to adapt the heuristic to allow for asynchronous dynamics. In the adapted version, if a gene’s value does not match the output expected by the values of its regulators, but it is consistent with the value of the gene in the previous time point, then it is no longer flagged as an inconsistency. Additionally, when fixing inconsistencies by performing a pass over trajectories and building a set of logic functions, functions are only updated when their target genes change their values between consecutive time steps. With these changes, the heuristic can be applied to asynchronous trajectories, or a combination of steady states and such trajectories.

As a final note, the heuristic described in 1 is in fact a family of heuristics. The order in which Boolean values are flipped in step 4 does not have to be arbitrary, and different criteria for this order will result in different solutions. Similarly, there are multiple ways to select a set of regulators, and various ways to cluster the states. Different choices will result in different heuristics.

## 2.2 Computational Complexity

For a Boolean network with  $N$  genes, where each gene has  $K$  potential regulators, there are  $2^{K \cdot N}$  possible structures, and for each such structure on average about  $2^{N \cdot K/2}$  logics. For  $N=10$  genes and  $K=3$ , this gives  $35 \cdot 10^{12}$  possible networks. Since this makes brute-force searches impractical, in this section we discuss the relationship between input size and running time. In [27] the Vertex Cover problem is reduced to the network inference problem by mapping each vertex to a regulator, and each edge that it covers to a target. The network structure is assumed to be fixed, and the noise bits (represented by the  $B$  variables) are minimized. Since the resulting dataset consists of steady states, it applies to both synchronous and asynchronous networks. If we don’t assume that the network structure is fixed, we can simply duplicate each target and its incoming edges multiple times, such that the dataset will be large enough to derive the network topology. Hence, the problem described in this paper is NP-Complete. In practice, higher levels of noise will result in longer running times, whereas for noiseless datasets the minimal network can be found relatively fast. Interestingly however, the dataset constructed by the reduction in [27] can be modified not to contain noise. That is, once the network structure has been selected, none of the measured gene values needs to be changed. This can be done by changing every regulator value in one of the two experiments. In this modified reduction, the vertex cover is the set of nodes that correspond to regulators selected for the network structure. Hence, assuming  $P \neq NP$ , in the worst case the structure inference alone will require exponential time. Considering the fact that the computation of the optimal network is performed in-silico, we argue here that the investment of computational resources is worthwhile. Once a network has been inferred, many insights that could have taken a significant amount of wet lab work could be derived. The Simulation section describes the gains in accuracy that are obtained by solving this problem. It is also important to notice

that when using an ILP solver, the good part of the computation is devoted to proving the optimality of a solution after the optimal solution has been found, so often an optimal solution can be found even if a time limit is imposed.

## 2.3 Pseudo-time

Pseudo-time assignment, also known as trajectory inference, is the ordering of individual states, produced by scRNA-Seq experiments, into trajectories and steady states. The method described in the previous section can be adopted for this purpose, in a procedure that analogous to Expectation-Maximization. Initially, the reconstructed network is set to the model that contains all the candidate edges, with some initial logic. For example, if edge signs are available or estimated from correlations, a rule like "inhibitors win over activators" can be used. Next, using the initial network, network trajectories from the states corresponding to the observed Boolean states of the cells are generated: each trajectory is extended until an attractor is reached, and also includes all attractor states. Each observed Boolean cell state is then mapped to its closest state in the trajectories that were obtained. Contiguous sequences of states are assigned to the corresponding trajectories time points, where trajectories of length 1 are set as steady states. Using the assignment of pseudo-time, a new network is reconstructed from the data, and a set of de-noised cell states is obtained as part of this reconstruction. Each of these can be used as an initial state for the inferred network in order to generate trajectories, map the data to them, and obtain a new pseudo-time. Using the new pseudo-time, a network is fitted to the data. The process repeats itself until the value of the solution stops improving. In the EM analogy, the latent variables are the pseudo-times assigned to cell states, and the network edges and logic are the model parameters. To understand the procedure better under the framework of algorithmic complexity, recall that we start with a fixed network structure and order the states to fit its trajectories. For simplicity consider a single trajectory. As the number of states  $M$  increases, the possible ways to order the states increases but constitutes an increasingly smaller fraction of all possible datasets. Hence, for an arbitrary single network the order of the states would form a random sequence of Boolean vectors. For a network whose dynamics are related to those of the real network, we will obtain non-random sequences of consecutive states, such that if we account for noise we can infer the real network more closely. By repeating the process, we converge to the real network. Convergence depends on starting "close-enough" to the real network. For ease of reference we will refer to this procedure as TICO (Timeless Inference of Cell Ordering).

## 3 Results

### 3.1 Synchronized Cells

To test our method on real experimental data, we obtained the microarray dataset GSE49650 of synchronized yeast cells from the Gene Expression Omnibus. Preprocessing of the .CEL files was done using the `rma` function in the Bioconductor package `affy`, using default argument values. Additionally, every trajectory (time-series) of every

gene was smoothed using the R functions `smooth.spline` with `smooth` parameter 0.5 and then `approxfun` (in the stats package) with default argument values [28]. The x-coordinate values for the smoothing were the times at which the measurements were taken in minutes, and the y-coordinate values were the array intensities. We used the BASCA method as implemented in the R package `Binarize` [20], with default arguments, for mapping from continuous to Boolean values. Every trajectory was binarized separately. We used the yeast cell cycle model from Cho et al. [29], with the edges as the candidate regulatory connections. Complexes were modeled using the expression of one of their genes. After fitting the model, the percentage of mismatches was 16.3%. In Cho et al., edges were associated with activation or repression activity. Compared to the inferred logic tables, all the edges agreed on the sign with Cho et al. This result is detailed in Table 1. The inferred logic provided information about combinatorial regulations, i.e. what is the joint effect of multiple regulators. This is illustrated in Figure 1 for two of the targets. Figure 2 shows an inferred trajectory for one of the time-series, and the original continuous levels for two of the genes. Interestingly, genes can be separated into those that are active at early or late cell cycle stages. More specifically, *CDH1*, *MCM1*, *SWI4* and *CLN3* are active at early time points, in accordance with their association with the G1 phase [29]. The other genes are active at later time points, in accordance with their association with S/G2/M. It should be noted that not every candidate interaction was inferred. The reason for this is that either the dataset size is too small for obtaining reliable predictions, or that the interactions occurring in the dataset do not capture all the candidate interactions. Perturbing a single gene in the steady state generates an oscillation that settles back to the steady state.

### 3.2 scRNA-Seq

Next, we applied the pseudo-time inference algorithm (TICO) to the HSC network of Bonzanni et al. [30], using GEO dataset GSE75478 [31], which profiles human hematopoietic stem cells in early differentiation from the bone marrow of two individuals. The raw data was preprocessed using the R package `scuttle`, followed by library-size normalization and log transformation. Binarized values were obtained using the BASCA method [20] from the R package `Binarize`, with default arguments. As an initial network for the pseudo-time inference algorithm, we used the logic published in Bonzanni et al. For validation, we used the CD38 measurements for individual cells, as CD38 is a marker for differentiation and therefore expected to increase along differentiation trajectories.

The binarized cell states were mapped to their closest counterparts in the network trajectories for the initial and inferred networks, and Pearson correlation and the corresponding correlation test p-values were computed between CD38 measurements in consecutive states, and thus correctly describes the phenotype. As shown in figure 3, correlation values from the inferred model were higher, with fold changes of 1.7 and 2.6 for individuals 1 and 2, respectively. A positive correlation corresponds to increase in CD38 levels as cells progress in their trajectories. The corresponding p-values were also lower, and significant for the inferred networks -  $8 \cdot 10^{-4}$  for individual 1 and  $1.24 \cdot 10^{-6}$  for individual 2. The  $\log_{10}$  ratio of the p-values obtained for the inferred and the initial networks are displayed in figure 3.

### 3.3 Simulation

In order to evaluate the accuracy of our inference algorithm, we generated 20 random networks using the R package BoolNet [32], and generated both steady-states and trajectories to test the algorithm and other methods that use steady states or trajectories. The trajectories include asynchronous updates and noise, where as steady states do not, as by definition they cannot include asynchronous updates. For tools that require continuous data, Boolean 0 and 1 are mapped to normally distributed continuous values -1 and 1, respectively, with standard deviation 0.25. The length of each trajectory depends on the network - the simulation includes all trajectory states until a state reoccurs.

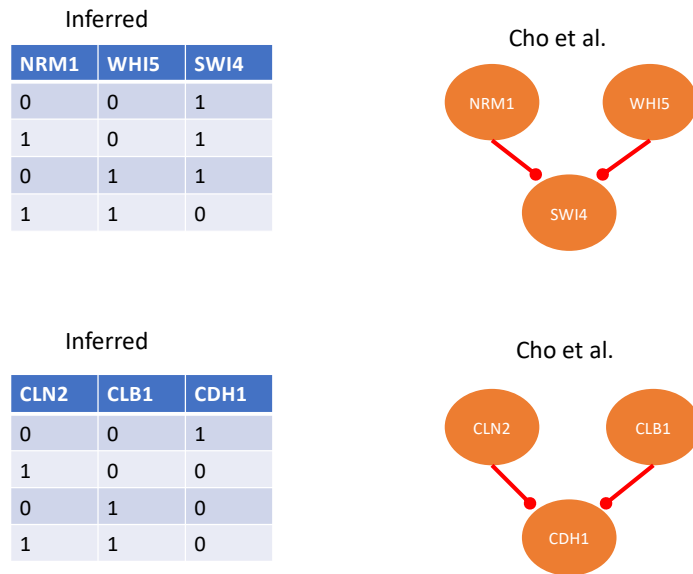
The other tools that we used are CLR [33], ARACNE [34], GENIE3 [35], BESTFIT [36] and GENENET [37]. As can be seen in figure4, our approach (denoted as MEDSI) is significantly more accurate than other methods. This boost in accuracy is likely due to the fact that our approach takes into account the computation performed by the networks, which allows a much more efficient separation between data and noise.

**Table 1** Inferred Edge Effects

Source	Target	Cho etl al.	GSE49650
MCM1	CLN3	activation	activation
SWI5	CLN3	activation	activation
NRM1	MCM1	repression	repression
CLN3	WHI5	repression	repression
CLN2	CDH1	repression	repression
CLB1	CDH1	repression	repression
SWI4	CLN2	activation	activation
NRM1	SWI4	repression	repression
WHI5	SWI4	repression	repression
CDH1	NRM1	repression	repression
CDH1	CLB1	repression	repression
CDH1	NDD1	repression	repression
NDD1	SWI5	activation	activation

## 4 Conclusion

In this work we presented a novel network-inference methodology that finds an optimal solution with respect to minimizing spurious fits, and can account for asynchronous updates in network dynamics. Our methodology is implemented in software and publicly available for the community. It can greatly enhance researchers' ability to understand their data in the context of a regulatory network. The experimental datasets that we analyzed exhibit asynchronicity and our algorithm was able to successfully infer the regulatory interactions. Based on this result, we believe that the algorithm is applicable to a broad range of high-throughput datasets. Several objectives are left for future work: First, additional heuristics should be developed



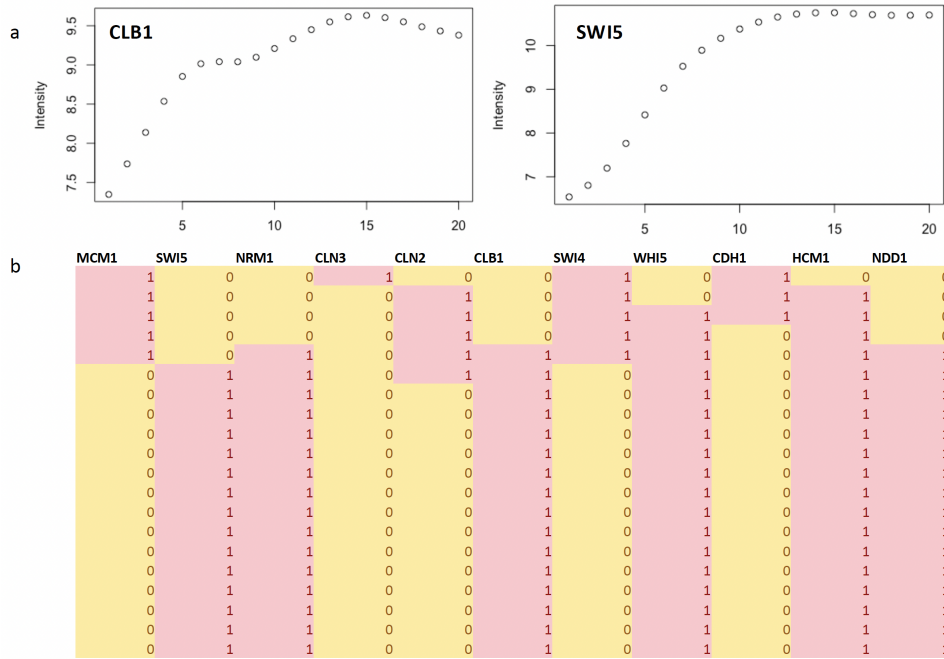
**Fig. 1** Regulations in Cho et al [29] and inferred combinatorial regulations

and studied, as the problem it addresses is likely to present a variety of challenging instances. Second, the topic of binarization of continuous or discrete data into Boolean values should be further pursued to obtain better understanding of current experimental technologies. Finally, combining different types of biological networks into a single model can provide broader insights into cellular function and should be studied using the method described in this work.

## Declarations

### 4.1 Competing Interests

The authors declare that they have no competing of interests.



**Fig. 2** Inferred Trajectory from the data of GSE49650, and the corresponding continuous values of two genes

## 4.2 Data Availability

An implementation of the method described in this work can be found at <https://github.com/karleg/MEDSI> The data is used in this work is available publicly at the Gene Expression Omnibus at <https://www.ncbi.nlm.nih.gov/geo/>

## 4.3 Ethics Declaration

Not applicable.

## 4.4 Consent to Publish Declaration

Not applicable.

## 4.5 Consent to Participate Declaration

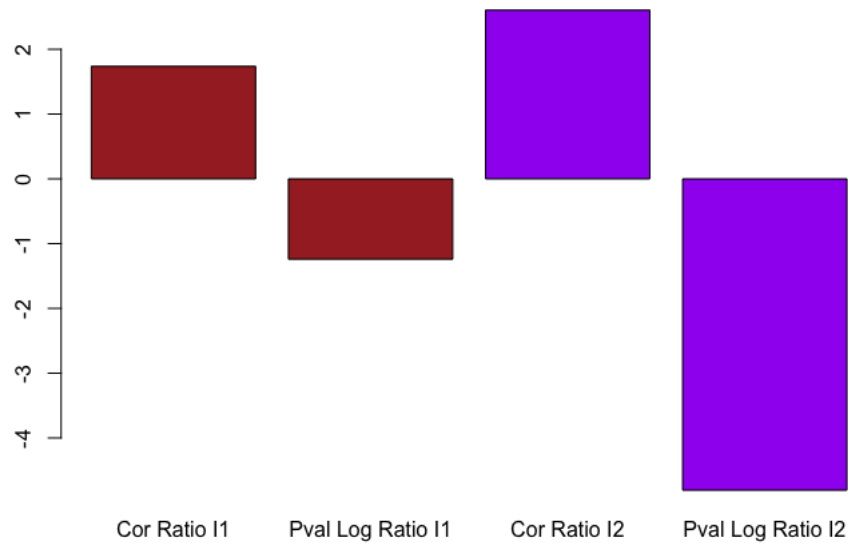
Not applicable.

## 4.6 Author Contributions

G.K. performed all the work described in this manuscript.

## 4.7 Funding

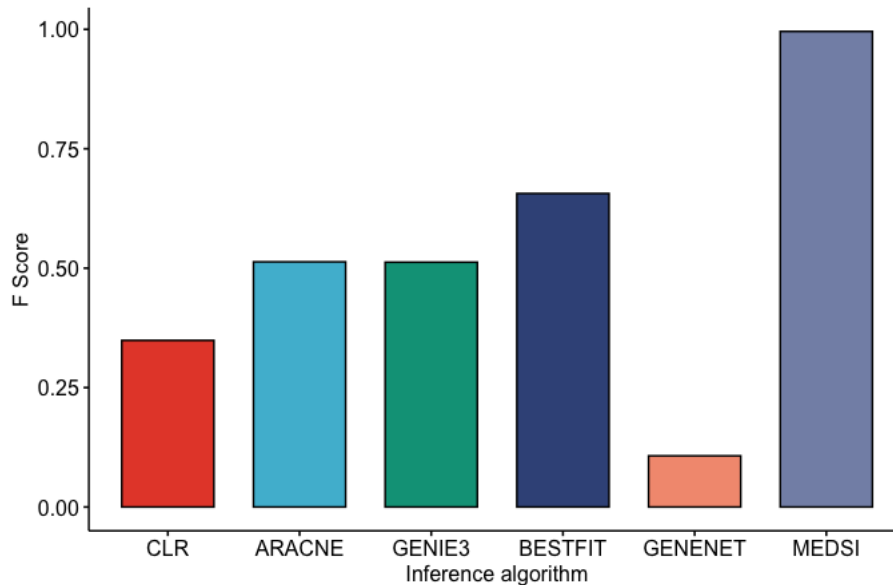
There is no funding to declare. =====



**Fig. 3** After vs. before inference correlation fold-change and p-value  $\log_{10}$  fold-change for CD38 measurements of adjacent trajectory cells, for two individuals I1 and I2

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**Fig. 4** True-positives (TP) and false-positives (FP) for TICO (blue) and SCORPIUS+bestfit (red)

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