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Network inference from single-cell data using multivariate information measures

Thalia E. Chan [1,†], Michael P.H. Stumpf[1,2,†,*], Ann C. Babtie[1,†,*]

1 Centre for Integrative Systems Biology and Bioinformatics, Department of Life Sciences, Imperial College London, London SW7 2AZ, UK
2 MRC London Institute of Medical Sciences, Hammersmith Campus, Imperial College London, London W12 0NN, UK
†All authors contributed equally
** m.stumpf@imperial.ac.uk, a.babtie@imperial.ac.uk*

Abstract

Gene expression is robustly controlled in space and time by networks of transcriptional regulators. Single cell data now allow us to probe and map these networks and cellular transcriptional states in unprecedented detail, but the heterogeneity and large sample sizes that characterise these data pose new analytical challenges. Here we develop an algorithm that exploits these features to infer gene (co-)regulatory networks from single cell data using multivariate information measures. Information theory allows us to detect complex non-linear relationships and explore statistical dependencies between multiple genes in detail. We thoroughly evaluate the performance of our algorithm in comparison to existing methods, demonstrate the benefits of considering multivariate information measures, and illustrate its application to several datasets. We provide guidelines for the use of information theoretical based algorithms, and software implementing our method to enable the identification of putative functional relationships and mechanistic hypotheses from single cell transcriptomic data.

Introduction

Precisely controlled patterns of gene expression are essential for the survival and reproduction of all life-forms. Development provides the canonical example, where changes in gene regulation determine the path by which from a single fertilised egg cell emerges a complex multicellular organism. Intricate networks of transcriptional activators and repressors have evolved to regulate the spatial and temporal expression of genes, enabling organisms to adjust transcription levels in response to environmental, developmental and physiological cues (Trapnell et al. 2014, Harrington et al. 2014, Rué et al. 2015, Moris et al. 2016, Gouti et al. 2015, Göttgens 2015). Elucidating the structure of such gene-regulatory networks (GRNs) has been a central goal of much recent systems biology research (De Smet et al. 2010, Oates et al. 2012, Thorne et al. 2012, Thorne et al. 2013, Siegenthaler et al. 2014, Oates et al. 2014, Huang et al. 2014, Young et al. 2014).

The structure of GRNs alone does not fully constrain their function (Ingram et al. 2006), but it serves as an important starting point for further analysis. The simplest mathematical representations of GRNs are static, undirected graphs, where each node represents a gene, and edges depict relationships between transcription factors and their targets. Although by this definition the GRN

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encapsulates every transcriptional regulatory relationship that could occur within a given organism, it is not the case that every possible interaction does occur in every cell — for example, the downstream interactions of a transcription factor only occur when it is expressed — hence we can define subsets of the GRN that are active in particular cells and contexts. The structure and dynamics of these active GRN subsets give rise to distinct mRNA expression profiles, and it has been suggested that characteristic expression profiles in different cell types (and under different conditions) result from different stable states of the GRN (Clevers et al. 2017, Huang 2010, Moris et al. 2016, Moignard et al. 2015).

The introduction of efficient high-throughput expression assays has driven interest in *network inference* methods that apply statistical approaches to identify likely regulatory relationships between genes based on their expression patterns. In addition to correlation-based networks (perhaps the simplest way of identifying putative relationships), Gaussian graphical models, (Dynamical) Bayesian networks, regression analyses, and information theoretical approaches have been used for network inference from population level data (Penfold et al. 2011, Penfold et al. 2015, Bonneau et al. 2006, Margolin et al. 2006a, Villaverde et al. 2013, Villaverde et al. 2013, Liang et al. 2008, Madar et al. 2010, Hill et al. 2012, Lèbre et al. 2010, Beal et al. 2005, Schäfer et al. 2005, Vinciotti et al. 2016). Combining multiple inferred networks to form a community or ensemble prediction often confers slight but consistent improvements in the quality of the predicted network (Hill et al. 2016, Marbach et al. 2010, Marbach et al. 2012), but how to best combine and weight different methods to form a consensus prediction is poorly understood. Given our current understanding, a reasonable approach to generate such ensemble predictions would be to include information derived using different classes of inference algorithms (since these are known to show different biases (Marbach et al. 2012)) but also to ensure that within each class we develop the best performing algorithm based on a given statistical methodology.

More recently, the increasing availability of single cell expression data has led to the development of several computational and statistical approaches aimed at gaining new insight into cell fate decisions and transitions between cell states (Pina et al. 2015, Moignard et al. 2015, Rué & Martinez Arias 2015, Moris et al. 2016, Bendall et al. 2014, Trapnell et al. 2014). Identifying the associated changes in transcriptional state, and regulatory interactions that contribute to controlling these processes are key aims of many single-cell transcriptomic studies (Kharchenko et al. 2014, Ocone et al. 2015, Moignard et al. 2015, Pina et al. 2015, Trapnell et al. 2014, Bendall et al. 2014). Several *pseudotemporal ordering* algorithms have been developed that aim to place cells in an inferred temporal order based on similarities in their transcriptional states (Trapnell et al. 2014, Bendall et al. 2014, Haghverdi et al. 2016, Reid et al. 2016, Setty et al. 2016), since true single-cell temporal data (where large numbers of genes are assayed) are not feasible to collect at present. These methods make strong assumptions about developmental processes (that

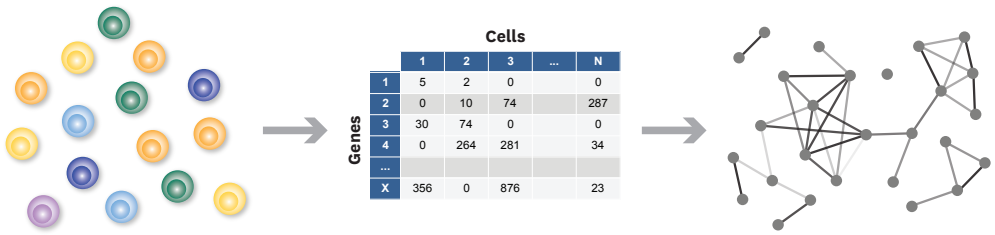

Figure 1: ***Network inference from single cell data.*** *Single cell transcriptomic data quantify mRNA species present inside individual cells. By considering pairs (or triplets, quadruplets,* etc.*) of mRNA species we can test for statistical relationships among them. These dependencies may reflect coordinated gene expression of these pairs (or groups) of genes, resulting from gene regulatory interactions or co-regulation. Once such sets of genes that jointly change in expression are known, other statistical, bioinformatic, or text-mining analyses can be used to identify likely transcriptional regulators for these sets of genes. By iterating such* in silico *analyses with further, targeted experimental studies we can, in principle, build up a representation of the gene regulatory network.*

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have been questioned (Moris et al. 2016)) and, in most cases, uncertainties in the inferred order are likely to affect and bias downstream analyses. Network inference methods, in contrast, explore statistical dependencies between genes (from the observed distributions of expression levels across a given population of cells) and identify those that may be indicative of functional relationships, without necessarily making such strong assumptions about the nature of cell transitions. Information theoretical measures, in particular, are particularly parsimonious in the assumptions that they make(Kinney et al. 2014), compared to e.g. ODE-basd regression approaches or simple graphical models. However, network inference using single cell data (Fig. 1) remains relatively unexplored with only a few notable examples, e.g. (Ocone et al. 2015, Moignard et al. 2015, Filippi et al. 2016), extending beyond simple correlation-based analyses (Bacher et al. 2016, Moignard et al. 2013, Kolodziejczyk et al. 2015, Pina et al. 2015, Stegle et al. 2015). This is perhaps because these datasets introduce new challenges for using and interpreting the results of existing methods: statistical challenges due to technical noise and heterogeneity and analytical challenges due to the presence of heterogenous subpopulations.

Single cell data are are notoriously complex and their analysis fraught with a host of potential problems (Stegle et al. 2015, Pierson et al. 2015, Grün et al. 2015, Bacher & Kendziorski 2016, Liu et al. 2016, Buettner et al. 2015): technical noise is difficult to distinguish from genuine biological variability, and relationships between gene expression vectors are complex and non-linear. Information theory offers an ideal solution by providing a set of measures, chiefly among them *mutual information* (MI), that allow us to characterize statistical dependencies between pairs of random variables without making assumptions about the nature of the dependencies (Cover et al. 2012, Mc Mahon et al. 2014). MI has considerable advantages over simpler measures such as (Pearson) correlation, as it is capable of capturing non-linear dependencies, and reflecting the dynamics between pairs or groups of genes (Mc Mahon et al. 2015, Uda et al. 2013). Calculating MI involves estimating pairwise joint probability distributions, generally requiring density estimation (Kraskov et al. 2004, Steuer et al. 2002) or data discretization, and the accuracy of these estimates depends on the sample sizes.

With single cell datasets, sample sizes are becoming sufficiently large to estimate probability distributions between more than two variables, allowing us to capitalise on recent developments in multivariate information (MVI) theory. Quantifying the information between three or more variables has long been problematic, and the most widely-used measures, such as interaction information, are known to have serious flaws (Timme et al. 2014). The recently-introduced partial information decomposition (PID) both explains and solves its predecessors' problems and provides a meaningful measure of MVI (Williams et al. 2010); PID and other measures are described in detail in Box 1.

Here we develop a novel GRN inference algorithm that applies PID to triplets of variables to generate undirected networks highlighting putative functional interactions between genes, and show that our approach outperforms algorithms based on pairwise MI. We demonstrate that the larger sample sizes of single cell data are vital for our method and that they profoundly improve the performance of information-based methods in general; continuing advances in single cell technologies will allow further increases in sample sizes (Macosko et al. 2015, Klein et al. 2015). Given the clear advantages of information measures for single cell data, we further demystify their use by providing analysis-based guidance for choosing an appropriate discretization algorithm and probability distribution estimator, along with a fast, open-source software package that implements the alternatives.

A further complication of single cell data is the biological heterogeneity found within certain cell populations (Grün & van Oudenaarden 2015, Stegle et al. 2015, Bacher & Kendziorski 2016, Liu & Trapnell 2016, Buettner et al. 2015). It is only possible to detect statistical relationships (interpreted as putative functional relationships) when there is variability in the data, and population-based studies introduce this variability by observing cell populations under different conditions, e.g. using time series or perturbation studies (Marbach et al. 2010, Marbach et al. 2012, Penfold & Wild 2011, Oates & Mukherjee 2012). It is worth emphasising that in these studies, certain edges of the GRN would not be inferred: those that are not active in the populations studied, and those that are active but with no variation; however, this is not necessarily problematic since those re-

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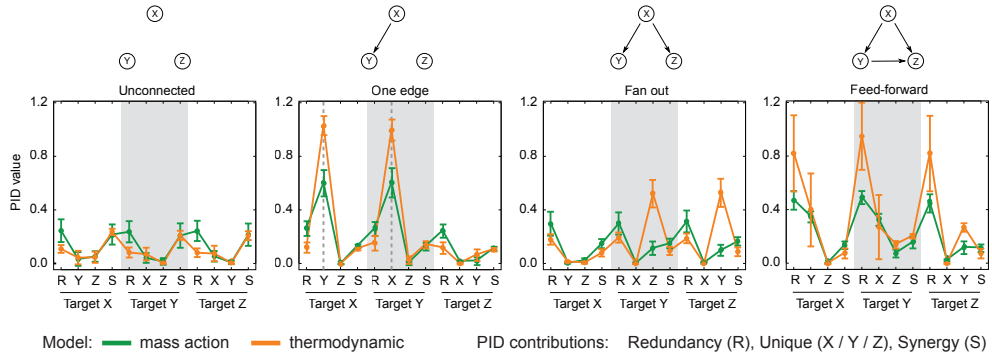

Figure 2: ***PID profiles for 3-gene networks*** *Mean PID values for 3-gene networks with different topologies. PID values are calculated using data simulated from 3-gene networks with the topologies illustrated above each plot; the models used for simulation assumed mass action (green) or thermodynamic (orange) kinetics. Each line graph shows the mean PID values calculated from simulations using different initial conditions (error bars indicate 1 s.d.), with the horizontal axis labels indicating the PID contribution, e.g. the first four values show the PID values with gene X as the target, consisting of the redundancy (R), unique contributions from gene Y (Y) and gene Z (Z), and the synergistic contribution (S). The vertical dashed grey lines in the 'One edge' plot indicate the unique PID values that are used as the basis for our inference algorithm. Here, all regulatory interactions are assumed to be activating, the additional stimulating ligand targeted gene X, and the values indicated are the mean PID values calculated from five sets of simulations (with different randomly sampled initial conditions); results obtained with models that include both activating and inhibitory regulation are shown in Fig. S1.*

lationships that do vary between the investigated conditions are of the most interest. Single cell data, by nature, offer a greater degree of variability — while we can collect time-series data, even data collected at a single timepoint will contain variability due to i) asynchrony of cells within a population (in terms of progression through a biological process), and ii) biological heterogeneity and often the presence of multiple cell (sub)types.

We emphasise the importance of considering these different sources of heterogeneity so that we can take advantage of the variation of interest — e.g. that associated with progression through a biological process such as differentiation. We demonstrate how our inference method can be combined with existing computational and statistical methods (e.g. clustering and dimensionality reduction) to infer networks from carefully-chosen subsets of single cell data in order to address particular questions about cellular processes. We consider three single cell transcriptomic datasets here and additionally refer to a related manuscript (Stumpf et al. 2017), in which we use our framework to infer changing regulatory sub-networks over the course of neural progenitor development in mouse embryos, and to suggest candidate genes for maintaining cellular states and driving state transitions.

In this paper, we develop a novel inference algorithm based on the MVI measure PID and use extensive analyses of *in silico* data to demonstrate i) the improvement over existing algorithms and ii) the suitability of this method for analysing single cell data, before illustrating its application to several experimental datasets. Such *in silico* analyses are critical for quantitatively assessing network inference approaches as, unlike with real biological systems, we have knowledge of the 'true' GRN underlying the observed data. We thoroughly explore the factors that influence algorithm performance — both in terms of the nature of the dataset and choices about data processing — in order to provide evidence-based guidelines for the use of information theoretical based methods for network inference. Finally, we provide a fast, open-source implementation of our methods to enable easy application to other single cell datasets.

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Box 1: Information theoretic measures

The entropy, $H(X)$, quantifies the uncertainty in the probability distribution, $p(x)$, of a random variable X. For a discrete random variable, the entropy is given by,

$$H(X) = -\sum_{x \in X} p(x) \log p(x), \tag{1}$$

which is maximal for a uniform distribution. If we consider the mRNA expression level of a gene to be the variable X, then a gene that is expressed differently across a set of cells will have a higher entropy than a more consistently expressed gene. When considering the relationship between X and a second random variable, Y, we quantify the information that one variable provides about the other using the MI,

$$\begin{aligned} I(X;Y) &= \sum_{x \in X} \sum_{y \in Y} p(x,y) \log \left(\frac{p(x,y)}{p(x)p(y)} \right) \\ &= H(X) + H(Y) - H(X,Y). \end{aligned} \tag{2}$$

This quantifies the difference between the joint entropy, $H(X,Y)$, and the joint entropy assuming independence of X and Y, $H(X) + H(Y)$, and thus provides a non-negative, symmetric measure of the statistical dependency between the two variables. For a pair of genes with more co-ordinated expression, their observed joint entropy will be lower, and hence they will have higher mutual information. Given a third variable, Z, the conditional mutual information (CMI),

$$I(X;Y|Z) = H(X,Z) + H(Y,Z) - H(X,Y,Z) - H(Z), \tag{3}$$

quantifies the information between X and Y given knowledge of Z. This tells us the extent to which knowing the expression of one gene additionally informs us about the expression of a second gene, given that we already know the expression of a third gene.

A number of MVI measures have been defined that aim to quantify the statistical dependencies between three or more variables, but there is little consensus as to the most appropriate metric (Timme et al. 2014). Arguably the most widely used is interaction information (McGill 1954), which for three genes quantifies the extra information between any two of the genes X, Y and Z, when the third is known compared to when it is not known,

$$II(X;Y;Z) = I(X;Y|Z) - I(X;Y) = I(X;Z|Y) - I(X;Z) = I(Y;Z|X) - I(Y;Z). \tag{4}$$

Interaction information has received much criticism since it can be (i) zero between dependent variables, when MI and CMI are equal but non-zero and (ii) negative, when MI is greater than CMI. The problem is that, despite its name, interaction information is not a quantity of information between a set of variables; rather it quantifies the balance between MI and CMI. In fact MVI is difficult to summarise with a single quantity, because there are different ways in which information can be shared by three or more variables.

An important recent development in information theory is the introduction of partial information decomposition (PID) (Williams & Beer 2010). In the three variable case, PID considers the information provided by a set of *source* variables (or genes), $S = \{X, Y\}$, about another *target* variable, Z, partitioned into redundant, synergistic and unique information. Redundant information is the portion of information about Z that can be provided by either variable in S alone; the unique contribution from X (or Y) is the portion of information provided only by X (or only Y); and the synergistic information is the portion of information that is only provided by knowledge of both X and Y. Thus the PID between the set S and the target variable Z is

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equal to the sum of the four partial information terms,

$$\begin{aligned} I(X,Y;Z) &= \text{Synergy}(Z;X,Y) + \text{Unique}_Y(Z;X) \\ &\quad + \text{Unique}_X(Z;Y) + \text{Redundancy}(Z;X,Y), \end{aligned} \tag{5}$$

where $\text{Unique}_Y(Z;X)$ is the unique information between source variable X and target variable Z when the other source variable is Y. In the context of gene expression, the terms 'source' and 'target' do not imply any mechanistic assumptions; rather, they refer to quantifying to what extent knowledge of the source genes inform us about the target gene.

To calculate the PID terms, the redundant information is first calculated using the specific information, I_{spec}, which quantifies the information provided by one variable about a specific state of another variable (Deweese et al. 1999, Timme et al. 2013). The 'state' of a gene in a given cell refers to which discrete bin its mRNA level falls within, once we have discretized the expression data. If we consider the information provided by X about state z of variable Z,

$$I_{\text{spec}}(z;X) = \sum_{x \in X} p(x|z) \left(\log \left(\frac{1}{p(z)} \right) - \log \left(\frac{1}{p(z|x)} \right) \right). \tag{6}$$

If we consider $S = \{X, Y\}$ and a target variable Z, the redundant contribution is calculated by comparing the amount of information provided by each variable within S about each state of the target Z,

$$\text{Redundancy}(Z;X,Y) = \sum_{z \in Z} p(z) \min_S I_{\text{spec}}(z;S). \tag{7}$$

The unique information terms can be calculated from the redundant information and the pairwise MI, via the relationship,

$$I(X;Z) = \text{Unique}_Y(Z;X) + \text{Redundancy}(Z;X,Y). \tag{8}$$

In other words, the pairwise MI between two variables can be partitioned into a redundant and unique component, given a third variable; it is this relationship that we exploit further in *Results*. We note that although PID is not a symmetric measure, $\text{Unique}_Y(Z;X)+\text{Redundancy}(Z;X,Y)$ will be equal to $\text{Unique}_Y(X;Z) + \text{Redundancy}(X;Z,Y)$ because MI is symmetric.

Finally the synergistic information can be calculated via the interaction information (Eq. 4), which turns out to be the difference between the synergistic and redundant information:

$$II(X;Y;Z) = \text{Synergy}(Z;X,Y) - \text{Redundancy}(Z;X,Y). \tag{9}$$

Results

PID profiles in synthetic data

We first investigate the usefulness of PID for inferring network edges using data generated from *in silico* models. We use stochastic simulations from simple directed 3-node networks of varying topologies, and estimate PID values (redundant, synergistic and unique information, defined in Box 1) from these simulated data. Simulations were generated from two model definitions, based on thermodynamic or mass action kinetics, both commonly used in systems biology to represent gene regulation, as described in *Methods*. A distinctive pattern is apparent in networks with a single directed edge between two genes ('one edge' topology, Fig. 2) — the unique information between the two connected genes is notably higher than both the unique information between unconnected genes and the redundancy values between all three genes. With increasing numbers of edges within the network, this pattern is lost; this makes sense intuitively as with higher connectivity we expect to see increased synergistic or redundant contributions (Fig. 2 and S1). Note

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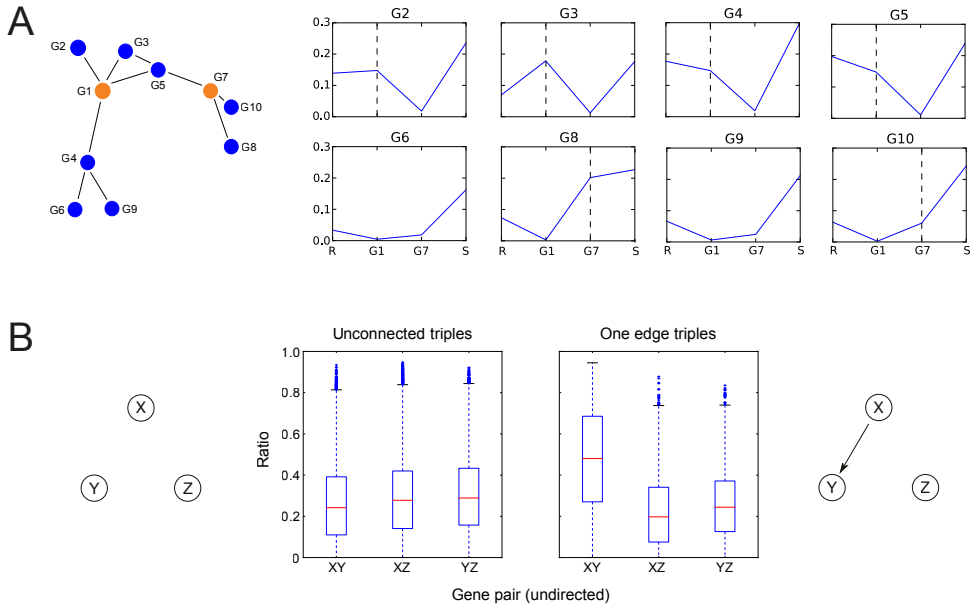

Figure 3: ***Demonstration of unique information in* in silico *networks.*** ***(A)*** *PID values are estimated from data simulated from a 10-gene* in silico *network (top) using* `GeneNetWeaver` *Each line graph shows PID values estimated using genes 1 and 7 as the sources, and each of the remaining genes in turn as the target (graph titles, GX, indicate the target gene). Four PID values are given in each graph — the redundancy (R), the unique information between gene 1 and the target (G1), the unique information between gene 7 and the target (G7), and the synergy (S). The mutual information between two genes is the sum of their unique information and the redundancy (Eq. 8). The ratio of the unique information to the mutual information tends to be higher between pairs of connected genes (dashed vertical lines indicate the unique contributions for connected genes).* ***(B)*** *Ratio of unique information to mutual information in triples with the two most common topologies, within the 50-gene network,* S. cerevisiae *1. For each gene triple of the 'unconnected' and 'one edge' topologies (see Table S1 for topology frequencies), we calculate the unique information values between each pair of genes and their mutual information. The ratios of unique information to mutual information are higher in general for the connected pair; the same pattern was observed in all networks.*

that the pattern can only be observed under simulation conditions that generate variability in the observed variables (i.e. statistical relationships are not detectable when the system is at steady state; Fig. S1).

To explore whether this pattern also occurs for triplets of nodes embedded in large networks, we consider time series expression data simulated from five different 50-gene networks generated by `GeneNetWeaver` (Schaffter et al. 2011). This software generates stochastic simulations from dynamical models that represent transcription and translation using a thermodynamic approach, with network structures that are inspired by known gene connectivity patterns in *E.coli* and *S.cerevisiae* (Fig. S3). Datasets of varying sizes (corresponding to differing numbers of cells) were generated by varying the temporal sampling regimes (see *Methods* for details). PID values are estimated from these data for every triplet of genes within the networks, and each triplet is classified according to its topology — six topological arrangements are possible given the model assumptions (maximum of one edge between each pair of nodes, no self-regulation, and no feedback loops). Mean PID values are calculated for each group, and the same distinctive pattern (high unique versus redundant contributions for connected genes) is apparent for triplets with a single directed edge (Fig. S2). As with the 3-node simulations, the pattern is lost in topologies with more connections; however, the 'unconnected' and 'one-edge' topologies are by far the most prevalent in all networks — comprising 64.4-93.2% and 6.3-29.7% of all triplets respectively (Table S1 and Fig S3).

Examining *in silico* data from a 10-gene network suggests that the relative size of the unique information compared to the redundancy — i.e. the proportion of MI accounted for by the unique

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contribution (Eq. 8) — is more informative than the absolute unique information (Fig. 3A). Using data from the 50-node networks, we confirm that this is the case more generally. We take all triplets of the 'unconnected' and 'one edge' topologies (with each gene being able to take part in multiple triplets); then for each triplet, treating each gene in turn as the target, we estimate (i) the redundant information between all three genes, (ii) the unique information between one source gene and the target and (iii) the unique information between the second source gene and the target. We plot the ratio of unique information to MI (the sum of unique and redundant information) for each pair of genes in each triplet and find that this ratio is higher in general between connected pairs (Fig. 3B).

Incorporating PID into an inference algorithm

In a network of n genes, given a pair of genes X and Y, there are $n-2$ gene triplets involving the pair. The MI between X and Y, $I(X;Y)$ is unaffected by the choice of the third gene, Z, because MI is a pairwise measure, but the unique information between X and Y, $\text{Unique}_Z(X;Y)$ varies depending on Z. Furthermore, the difference between $I(X;Y)$ and $\text{Unique}_Z(X;Y)$ is equal to the redundancy between all three genes (Eq. 8), meaning that we can regard the ratio $\text{Unique}_Z(X;Y)/I(X;Y)$ as capturing the proportion of MI that is accounted for by unique information between X and Y, as opposed to redundant information between all three genes. We note that the vast majority of all possible gene triplets in our *E.coli* and *S.cerevisiae* networks are of the 'unconnected' or 'one-edge' topology (Table S1), and that the ratio $\text{Unique}_Z(X;Y) : I(X;Y)$ is higher between connected pairs in a 'one edge' triple (Fig. 3). Therefore, we would expect that if X and Y are connected, then most of the triplets made with X, Y and all $n-2$ possible Z in turn, would be of the 'one edge' topology — and likewise if X and Y are unconnected, then most triples involving X and Y would be of the 'unconnected' topology — so $\text{Unique}_Z(X;Y)/I(X;Y)$ would in general be higher if X and Y were connected.

We define the *proportional unique contribution* (PUC) between two genes X and Y as the sum of this ratio calculated using every other gene Z in a network (where S is the complete set of genes),

$$u_{X,Y} = \sum_{Z \in S \setminus \{X,Y\}} \frac{\text{Unique}_Z(X;Y)}{I(X;Y)} + \sum_{Z \in S \setminus \{X,Y\}} \frac{\text{Unique}_Z(Y;X)}{I(X;Y)}; \tag{10}$$

this measure may be thought of as the mean proportion of MI between two genes X and Y that is accounted for by the unique information. Note that the PID unique measure is not symmetric, so for each pair of genes we consider each as the target in turn (hence we include both $\text{Unique}_Z(X;Y)$ and $\text{Unique}_Z(Y;X)$ terms in Eq. 10).

In our network inference algorithm (Fig. 4), the redundancy and unique information contributions are first estimated for every gene triplet, then the PUC is calculated for each pair of genes in the network (Eq. 10). Finding a threshold for defining an edge at this stage is problematic, because the distribution of PUC scores differ between genes (see Fig. S3), thus setting a global threshold for PUC scores across the whole network risks biasing the results by factors such as expression variability. This was previously observed with MI and led to the development of measures that take into account the network context, central to the *context likelihood of relatedness* (CLR) algorithm (Faith et al. 2007, Watkinson et al. 2009). A similar solution is employed here: an empirical probability distribution is estimated from the PUC scores for each gene, and the confidence of an edge between a pair of genes is given by,

$$c = F_X(u_{X,Y}) + F_Y(u_{X,Y}), \tag{11}$$

where $F_X(U)$ is the cumulative distribution function of all the PUC scores involving gene X (here, we assume either a Gamma or Gaussian empirical probability distribution).

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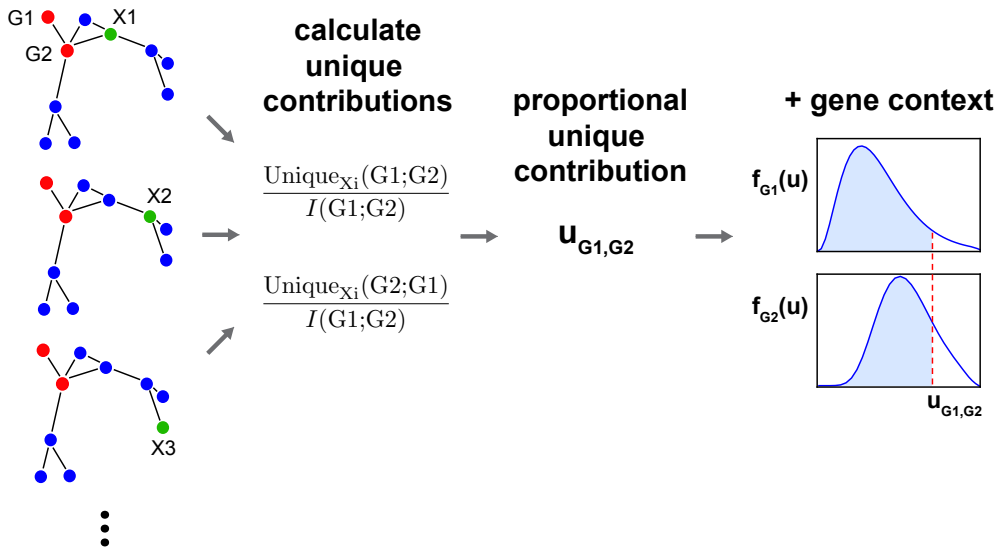

Figure 4: ***Schema of the PIDC inference algorithm.*** *PID values are estimated for every gene triplet (with each gene treated as the target gene in turn), and from these the PUC, $u_{X,Y}$, is estimated for every pair of genes. For each gene, X, an empirical distribution, $f_X(u)$, is estimated from its PUC scores with all other genes. The confidence of an edge between a pair of genes depends on the corresponding cumulative distribution functions, $F_X(u)$, for each gene within the pair (i.e. the blue shaded areas); these confidence scores are used to rank all possible network edges.*

Algorithm performance

We compare our new algorithm against several common information theoretic based network inference methods, and thus briefly summarise these approaches in Box 2. All these methods start from the pairwise MI matrix, and then use it in different ways. Even compiling the MI matrix is, however, fraught with potential problems: the manner in which data are treated (e.g. discretization), and the estimator used for the entropy and MI both affect the performance of the algorithms (Simoes et al. 2011, Zhang et al. 2015). When comparing different approaches it is therefore important to ensure that discretization and estimation of MI are done identically. Without this it becomes impossible to disentangle the relative strengths and weaknesses of the different approaches that are based on and interpret MI values. In the *Methods*, we discuss the different estimators and discretization approaches that we use and which are implemented in the `InformationMeasures.jl` package (see *Software*). Our comparisons with existing methods thus always start from the same MI matrix.

Undirected networks are inferred from *in silico* datasets (described in *Methods*) for five 50-gene networks and five 100-gene networks using ARACNE, CLR, MI (relevance networks), MRNET (Meyer et al. 2008) and the new PID-based algorithm, *PID and context* (PIDC). We also include results for the raw PUC score, without the network context step. Accuracy of the inferred networks is evaluated using the area under the precision-recall curve (AUPR) and the area under the receiver operating characteristic curve (AUROC), see *Methods* for definitions (Murphy 2012).

The raw PUC score outperforms the raw MI score and is closely matched by CLR, which outperforms the other MI-based approaches in agreement with the previous comparisons (Fig. 5) (Marbach et al. 2012). The PID-based algorithm PIDC, which combines the PUC measure with the network context, performs favourably compared to the other algorithms (Fig. 5), particularly in the larger networks. This effect is robust to simulated technical noise (Fig. S5C) and becomes more evident the larger the number of 'cells' in the dataset (Fig. 5); as real data become more accurate and sample sizes increase, we expect the performance of PIDC/PUC to improve as the estimation of 3-dimensional dependencies becomes more accurate. Also, unlike CLR it was designed to capture higher order dependencies and to distinguish between direct and indirect interactions (Williams

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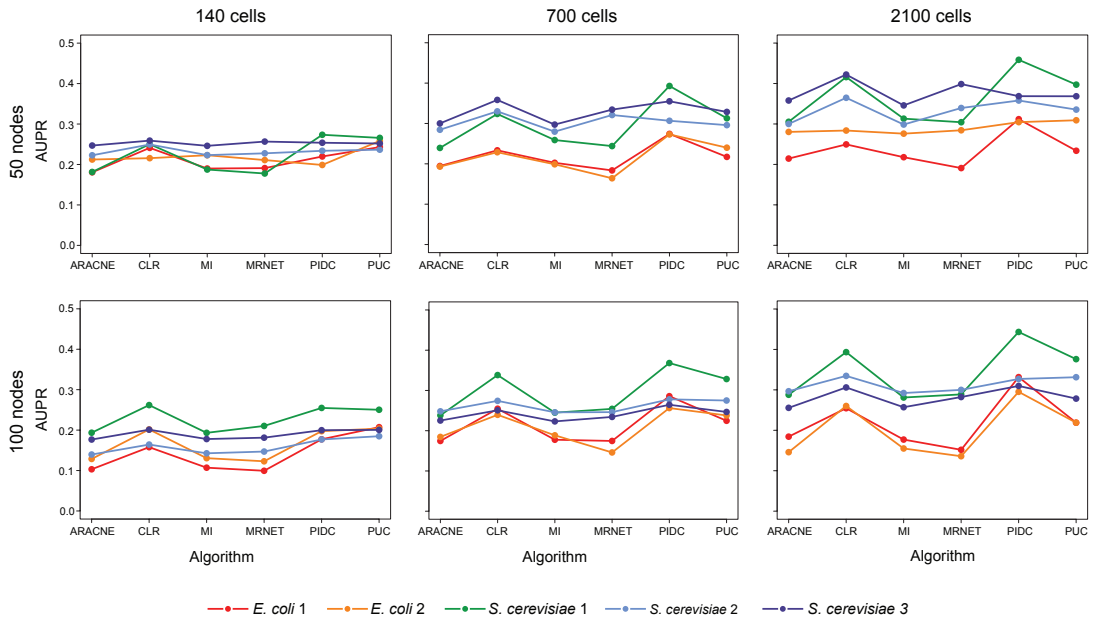

Figure 5: ***Performance comparison of information-theoretic network inference algorithms.*** *AUPR is calculated for several algorithms applied to ten* in silico *datasets, generated from five 50-node and five 100-node networks, using Bayesian Blocks discretisation and the maximum likelihood estimator. All algorithms performed better with larger datasets, and the PID-based algorithm PIDC and the raw PUC score performed well in general. The algorithms used are described in* Methods *and* Results, *with MI indicating the use of mutual information scores alone to rank edges (i.e. MI relevance network); the R package* `minet` *was used for the existing inference algorithms.*

& Beer 2010).

Box 2: MI-based algorithms

Relevance networks (Butte et al. 2000) use the MI estimates (or, in some cases, correlation) in order to detect edges. As there is no reliable universal way of determining the statistical significance of MI values, a threshold is typically chosen to determine which edges are present. This fails to account for the fact that MI may be increased for nodes X and Z even though they only indirectly interact via an intermediate node Y. The *Data Processing Inequality* (DPI) allows us to sort out some of these cases by virtue of the relationship

$$I(X;Z) \leq \min\left(I(X;Y), I(Y;Z)\right), \tag{12}$$

which holds whenever X, Y and Z form a Markov Chain. Post-processing of the MI values using the DPI is at the core of the popular *ARACNE* algorithm (Margolin et al. 2006a, Margolin et al. 2006b). Thresholds on the pairwise MIs are used to identify likely dependent pairs X, Z; MI values above the threshold are then considered with every possible other node Y in light of the DPI.

Given that MI values are affected by a number of factors, including especially the variability of each individual random variable, any global *a priori* threshold may be highly problematic: it will give rise to false positives as well as false-negatives. In the CLR algorithm (Faith et al. 2007, Watkinson et al. 2009) the MI between X and Z is considered against all MI values for pairings of X and Z with all other variables Y. Thus the threshold for each pair will reflect the

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variabilities of both genes, as well as their relative levels of statistical dependence on other genes. *MRNET* (Meyer et al. 2007) aims to identify a minimally redundant but maximally explanatory set of variables/predictors for each target gene X in a greedy manner.

There have been attempts of using conditional mutual information, interaction information, (4), or related concepts, for network inference (though not single cell data) (Watkinson et al. 2009, Villaverde et al. 2013, Villaverde et al. 2014, Liang & Wang 2008, Zhao et al. 2016). These would have to deal with the known difficulties (Timme et al. 2013) of interpretation (which do not arise in relation to PID-based measures), which may explain the lack of their widespread uptake.

Application to single-cell data

The extensive analyses using simulated data are necessary to validate our algorithm and provide quantitative comparisons with existing methods. When working with real experimental datasets, we of course do not know the true underlying network, and thus rely on identifying relationships that are consistent with our current biological knowledge about the systems we are studying. Here, we apply our algorithm to three published experimental datasets and, in a related manuscript (Stumpf et al. 2017), illustrate how it can be used as part of a thorough modelling analysis of neuronal differentiation of mouse embryonic stem cells.

Psaila *et al.* (Psaila et al. 2016) used single-cell quantitative PCR (sc-qPCR) to study megakaryocyte-erythroid progenitors (MEP). Their analysis revealed the existence of subpopulation structure in this class of cells: two groups of cells are primed preferentially for a particular cell fate — megakaryocytic (MK-MEP) or erythroid (E-MEP) — while a third group of multi-potent progenitors (Pre-MEP) retain some myeloid differentiation capacity (Fig. 6A). Here we apply our PIDC algorithm to their complete dataset and infer a candidate network that depicts statistical dependencies among the analysed genes (Fig. 6B). Given we are interested in genes involved in differentiation processes, we also consider networks inferred using overlapping subsets of the data and colour each edge in Fig. 6B according to their presence in these additional networks. Edges that are present in the network constructed using Pre-MEP & E-MEP cells, but not that based on Pre-MEP & MK-MEP cells, are coloured red (i.e. erythroid specific), and the reverse (megakaryocytic specific) are shown in blue (edges present in both, or just in the original complete network are shown in grey). Consistent with existing knowledge about these two lineages, we see a cluster dominated by blue edges that comprises known megakaryocytic genes (e.g. *Cd9*, *Lox*, *Vwf*, *Nfib*, *Cd61*, *Tgfβ1*) and a cluster with several red edges comprising known erythroid genes (e.g. *Cd36*, *Klf1*, *Lef1*, *Cnrip1*, *Tmod1*, *Ank1*, *Dhrs3*) (Psaila et al. 2016). Networks based on pairwise MI scores from the same data show skewed degree distributions with many nodes unconnected (Fig. S6A,B).

We next consider sc-qPCR data comprising expression measurements of selected genes during early embryonic development (from oocyte to 64-cell blastocyst stages) (Guo et al. 2010); Fig. 6C shows the resulting inferred network. We again infer additional networks using subsets of these data — here we use overlapping subsets of 'early' and 'late' cells to reveal any temporal dependencies in the detected interactions. A number of known relationships between genes are apparent in the network, e.g. upregulation of *Cdx2* and *Gata3* transcription factors (TFs) during the 8-cell to morula transition is identified as an edge in the 'early' network; while the co-expression of primitive endoderm specific TFs *Creb312* and *Sox17* is detected as an edge in the 'late' network (consistent with the appearance of distinct cell types, including primitive endoderm cells, in the blastocyst). A cluster of known pluripotency and reprogramming factors is also identified in the network (*Pou5f1*, *Nanog*, *Esrrb*, *Klf2* and *Klf4*) — *Sox2*, another key reprogramming factor, is not connected with these genes but is known to be upregulated later than the other factors (Guo et al. 2010).

Moignard *et al.* studied embryonic haematopoietic development, and used sc-qPCR data to develop a Boolean network model of the GRN underlying blood development (Moignard et al. 2015). In Fig. 6D we compare the networks inferred from these same data using PIDC and MI — we find the inferred PIDC network shares a higher number of edges with the Boolean network model, than the network constructed using MI values alone (see Fig. S6C for node labels). Although we of

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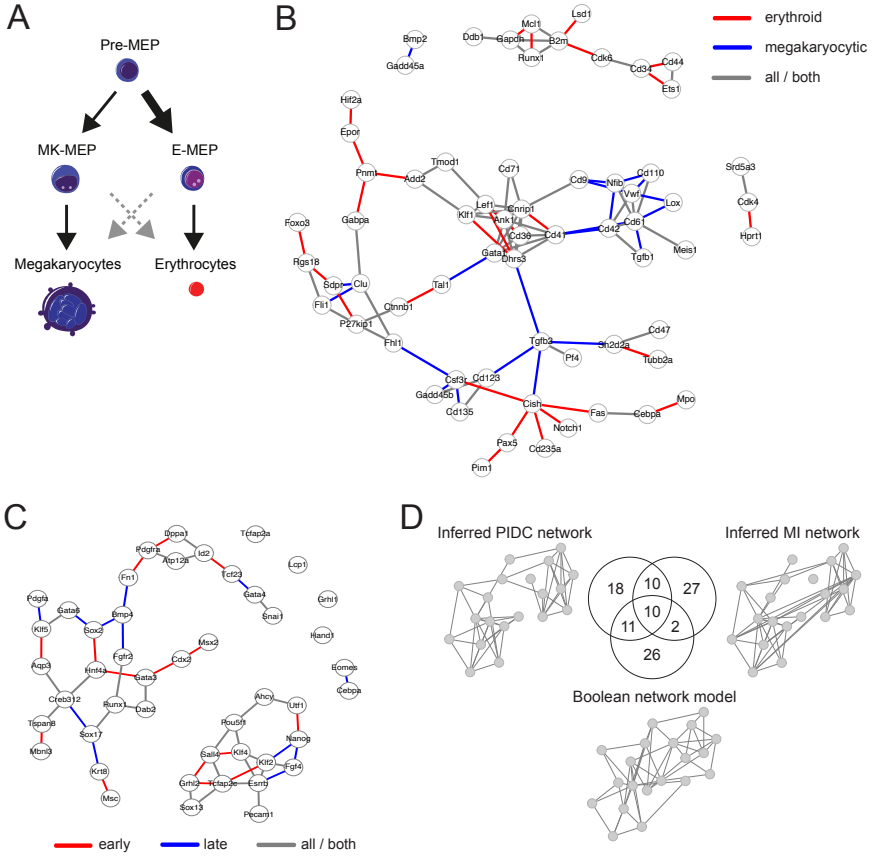

Figure 6: ***Application of the PIDC inference algorithm to experimental datasets. A)*** *Illustration of the relationship between the three subpopulations of MEP cells — 'Pre-MEP' cells are enriched for erythroid/megakaryocyte progenitors but still retain some potential to differentiate into other cell types (myeloid cells); 'E-MEP' and 'MK-MEP' cells are strongly biased towards erythroid and megakaryocyte differentiation respectively (for details see Psaila* et al.*).* ***B)*** *Network inferred using the PIDC algorithm from the complete set of data from Psaila* et al. *(the top 2.5 % of edges are shown; for clarity, only nodes connected by these edges are shown). Edge colours indicate whether these edges are also detected in networks constructed using subsets of the data (comprising data from Pre-MEP cells combined with either E-MEP or MK-MEP cells). Red edges indicate those that are present in the Pre-MEP & E-MEP network but not the Pre-MEP & MK-MEP network (i.e. erythroid specific), while blue edges indicate the reverse scenario (i.e. megakaryocytic specific); edges present in both networks, or only in the network constructed using all the data, are shown in grey.* ***C)*** *PIDC interaction network inferred using early embryonic development data (oocyte to E4.25 blastocyst stages). Graph edges indicate the top 5 % of putative interactions detected using the PIDC algorithm on the complete dataset. Networks is also inferred using two overlapping subsets of the data — an 'early' subset that includes all cells collected from oocyte up to 32-cell E3.5 blastocyst stages, and a 'late' subset including cells collected from 16-cell morula to 64-cell E4.25 blastocyst stages. Edge colours indicate temporal dependencies of the identified relationships; red indicates an edge ranks in the top 5 % of edges in the early network but not the late network, blue indicates the converse (late but not early), while grey indicates relationships without specific temporal dependencies (i.e. only present in the network constructed using the complete dataset, or present in both the early/late networks).* ***D)*** *Comparison of networks inferred using haematopoietic development data in Moignard* et al.*. Moignard* et al. *used single-cell expression data for 20 transcription factors to infer a Boolean network model of blood development; we show a simplified representation of their model, where nodes (genes) are linked by an edge if those genes are either directly linked, or linked via one Boolean operation or set of update rules (genes linked in their model via a chain of multiple sets of update rules are not connected here). We used our PIDC algorithm or MI alone to infer networks of putative interactions between genes using these same data, and compare the edges identified in each of these three networks (numbers of shared edges are indicated by the Venn diagram). For node labels, see Fig. S6C.*

course do not know the 'true' structure of the GRN in this case (this is only feasible when using *in*

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silico data), the Boolean model was shown to capture key cell states observed experimentally and generated several experimentally-validated predictions (Moignard et al. 2015), thus we use this as a benchmark to indicate the biological plausibility of our inferred networks.

In a related companion paper (Stumpf et al. 2017) we apply our PIDC algorithm to sc-qPCR data collected from cells undergoing differentiation from a pluripotent ground state towards a committed neuronal lineage, via a primed epiblast-like state. 547 cells were sampled in total at seven timepoints spanning seven days; we analysed expression measurements of 74 genes including known regulators of pluripotency and neuronal differentiation (see (Stumpf et al. 2017) for details). We first assign the cells to three robust groups that correspond with developmental stage (using k-means clustering), and then infer networks using data from all the cells, or from overlapping subsets containing cells at earlier or later stages of development. Comparing the networks obtained using different subsets of cells allows us to observe any temporal dependencies in the inferred interactions. Using an unsupervised community detection algorithm, we find that the network of inferred (co-)regulatory relationships contains several communities (or modules) of genes displaying high connectivity within each community. Three of these communities show distinct temporal dependencies in connectivity, and comprise genes known to play roles at different stages of differentiation. Our analysis thus allows us to identify modules of genes that undergo coordinated changes in expression as cells progress through development, and putative gene interactions that may be involved in regulating these transitions in cell state.

Guidelines & limitations

Any comparative analysis of information-based GRN inference algorithms is influenced by a number of decisions, in particular: (i) how the data are discretized, (ii) the choice of MI estimator, and (iii) the metric used to evaluate performance. We discuss the impact of each of these decisions and offer guidelines for future analyses.

The information measures described here all rely on estimates of discrete probability distributions, and yet mRNA expression data are continuous. Several algorithms and heuristics have been developed to discretize data and estimate empirical probability distributions from the resulting discrete frequencies. We investigated two methods for discretization, along with four MI estimators, as described in *Methods*.

All estimators produce fairly accurate estimates of joint entropies for up to two uniformly distributed random variables, but in higher dimensions performance varies according to the distribution (Tables S2 and S3), making it difficult to identify the most appropriate estimator for experimental data. Rank agreement between the estimators is good when the data are discretized using Bayesian blocks, however, diminishing the importance of the choice of estimator (Fig. S5). In light of these findings we advise using Bayesian blocks, and since the underlying distribution is usually unknown we favour the maximum likelihood estimator due to its simplicity.

The choice of discretization method and estimator influences the performance of the inference algorithms, with effects varying depending on the algorithm and on the true network (Fig. S5A and S5B). Sampling frequency and dataset size also have an effect, with performance increasing in line with data set size, and decreasing in larger networks and with a higher rate of technical drop-out errors (Fig. S5C). Due to the number of influential factors, we advise caution when interpreting the results of this or any such comparison as an exhaustive exploration of these factors is not feasible; however we note that the PIDC algorithm performs well in general across the many tested combinations of discretization methods, estimators and datasets.

The metric used to evaluate algorithms also affects the apparent performance of the inference algorithms, evident here in the higher scores for AUROC than AUPR (Fig. 5 and S5). This is a well-documented phenomenon, caused by the true negatives in a GRN vastly outnumbering the true positives, meaning that AUPR is the more meaningful measure, despite AUROC being more widely-used (Murphy 2012).

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Software

A new open-source package for estimating MVI measures is implemented in the Julia programming language (Bezanson et al. 2014). The package, named `InformationMeasures.jl` supports information measures such as entropy, MI, CMI, and PID; the maximum likelihood, Miller-Madow, Dirichlet and shrinkage estimators; and the Bayesian Blocks, uniform width and uniform count discretization methods.

Julia was chosen for its speed (Fig. 7A), clear mathematical syntax, growing availability of libraries and good integration with other languages. The existing `Discretizers.jl` package is used to implement the discretization methods; in some of our initial analyses we used the `AstroML` Python implementation of the Bayesian Blocks algorithm (Vanderplas et al. 2012, Scargle et al. 2013). In order to meet a wide range of requirements, the package can be used simply for discretizing data, or to calculate information measures using pre-discretized data or probability distributions that have been estimated elsewhere.

A Julia script implementing the PID-based network inference algorithm (PIDC) is available from our website. The algorithm has complexity $O(n^3)$ in the number of genes, therefore it is best suited for small to medium-sized inference problems (Fig. 7). The complexity in the number of cells depends on the discretization method: the recommended Bayesian blocks method scales less well than the uniform width method, but nevertheless produces results for several thousands of cells for a medium-sized network (a few hundred genes) within a practically useful timescale (Fig. 7B-C). When analysing larger datasets (e.g. as obtained using scRNA sequencing where data is available for thousands of genes), a subset of (up to hundreds of) genes should first be selected, e.g. based on prior knowledge of the specific system being studied (or using functional annotations of genes), or those that show high variability between cell states or samples. This not only makes the subsequent network inference analysis computationally tractable, but will also aid interpreting the results. As is the case for other network inference algorithms, genes with no variability in mRNA expression are uninformative, and should always be removed prior to analysis.

Discussion

Here, we have described a network inference algorithm based on PID (Williams & Beer 2010, Timme et al. 2014), an easily interpretable MVI measure that allows us to explore statistical dependencies between multiple genes in detail. In particular we demonstrate that the unique contribution (Eq. 5) to the MI between a pair of genes is particularly informative about direct relationships, as we might intuitively expect. When combined with information about the local network context of each gene, this provides a robust measure of putative functional relationships. We take gene context into account both by considering the ratio of unique information to MI (effectively normalising MI), and by using empirical distributions to identify the highest scoring edges for each gene (similar to CLR (Faith et al. 2007, Watkinson et al. 2009)).

Numerous algorithms have been developed to infer the structure of biological networks from observations of the constituent components. To thoroughly compare and quantify the relative performance of these methods we necessarily rely on *in silico* studies where we know the true network that generates the data; with experimental data, we of course only have our current knowledge about a given biological system, and the inherent uncertainties associated with that. Here, we rigorously analyse the performance of our method and other popular information theoretic based algorithms for network inference, and explore the factors that influence their performance in much greater depth than most existing studies. This is crucial to understand the relative advantages of different methods, explore their limitations, and thus gain confidence in our conclusions.

All the methods we consider rely on estimating entropies and probability distributions following discretisation of (continuous) transcriptomic data. Several algorithms exist to achieve these steps — with no consensus as to the best approach — but, like other studies (Simoes & Emmert-Streib 2011, Olsen et al. 2009, Hausser et al. 2009), we find these choices can greatly influence

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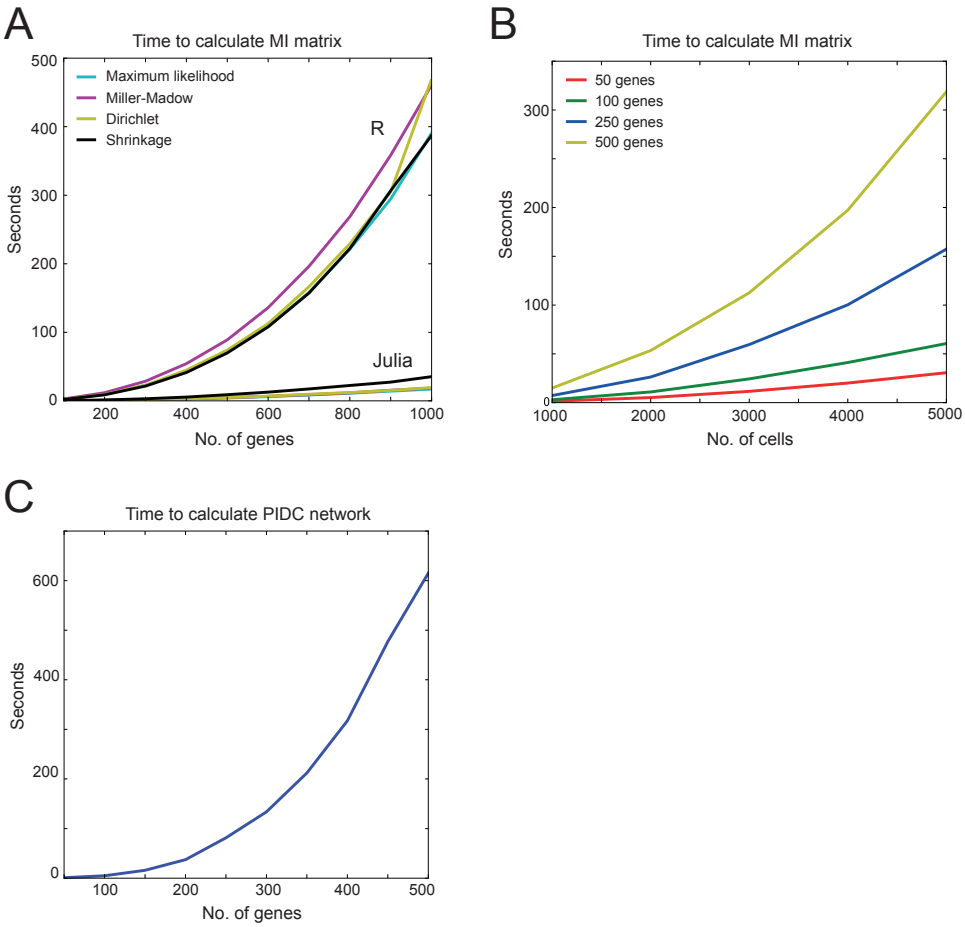

Figure 7: ***Speed of MI and PIDC calculations using the Julia programming language.*** ***A)*** *Comparison of the times taken to calculate a matrix of pairwise MI values using the R package* `minet` *(Meyer et al. 2008) and our Julia package* `InformationMeasures.jl`*. Input data were simulated expression values for up to 1000 genes, with 700 values per gene (equivalent to 700 cells, the same as our medium-sized* in silico *dataset). Data were discretised using the uniform width algorithm, because Bayesian blocks was not available via* `minet`*, and times were measured using inbuilt functions in R and Julia.* ***B)*** *Times taken to calculate the MI matrix in the Julia programming language with larger numbers of cells, for networks of different sizes. Data were discretised using the recommended Bayesian blocks algorithm, which has a much greater complexity than the uniform width algorithm, but produces better estimates.* ***C)*** *Time taken to infer networks of varying sizes using the PIDC algorithm implemented in Julia. Networks were inferred for simulated datasets of up to 500 genes, with 700 expression values per gene.*

performance (Fig. S5A,B). We find the choice of discretisation method to be particularly important with the best (and most consistent) performance achieved using Bayesian blocks. This is an adaptive algorithm that aims to find an optimal discrete representation for a given dataset, so it is perhaps unsurprising that this outperforms simpler, more naive approaches such as uniform width. Too often, the impact of these choices is not explored sufficiently when analysing information theoretical methods, despite how influential they can be.

The benefit of considering MVI measures — illustrated by the good performance of our algorithm throughout our extensive comparisons — is counterbalanced by the difficulty of accurately estimating higher dimensional probability distributions (Table S2). The increased performance afforded by our algorithm is particularly noticeable when analysing larger datasets (Fig. 5), and thus the large sample sizes provided by single cell data are critical to its success. As experimental technologies advance enabling more cells to be assayed (and potentially reductions in technical noise) we expect to further improve the accuracy of these multi-dimensional distributions and see associated gains in performance.

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The best performing algorithms, PIDC and CLR, both take gene context into account and aim to capture the most important interactions for each node, which of course influences the types of network structures inferred. The resulting graphs tend to be well connected, whereas MI relevance networks can produce graphs with more skewed degree distributions (e.g. Fig S6A,B) as a result of high-variability nodes predominating amongst the highest pairwise MI scores. This should be borne in mind when selecting inference algorithms, as depending on our beliefs about the structure of the true network, and the aims of our analyses, we may want to emphasise (or elucidate) different network features. Considering network context should provide an additional benefit when working with single-cell data, by helping to address the influence of potential confounding factors such as the cell cycle. Variation in cell-cycle stage is known to cause large-scale changes in cell transcriptional states (Buettner et al. 2015, Scialdone et al. 2015, McDavid et al. 2014) — and we would expect it to induce stronger statistical dependencies amongst the affected genes. However, using empirical distributions to take network context into account (as in PIDC and CLR) ensures that we identify the most important interactions for each node in turn, rather than the highest dependencies across the whole dataset, and this should act to partly mitigate the influence of any confounding factors which cause more global changes in transcriptional state that affect large numbers of genes in a coordinated manner.

In order to detect statistical dependencies between genes we require their expression states to be variable within the observed dataset. There are of course many sources of heterogeneity in single-cell data so it is important to consider the sources of variation that are of interest. For example, when studying developmental processes we should aim to analyse collections of data where we expect cell differentiation to be the major source of variation. We can make use of well-established methods for analysing the subpopulation structure of single-cell data — such as clustering, dimensionality reduction, and pseudotemporal ordering algorithms (Bacher & Kendziorski 2016, Stegle et al. 2015, Grün & van Oudenaarden 2015, Liu & Trapnell 2016) — to examine our data and e.g. identify distinct cell subtypes or alternative differentiation pathways. This allows us to select suitable subsets of the data to address our specific biological questions; we illustrate in the examples here how this can enable us to focus on functional relationships involved with different developmental transitions, or the relative timing of transcriptional changes. For example, in our related manuscript (Stumpf et al. 2017) we use clusters of developmentally-similar cells to examine how the activity of (co-)regulatory relationships changes during development, making initial steps towards defining mechanistic models of neural differentiation — the network structure and temporal information allow us to suggest candidate genes for maintaining cell states or driving developmental transitions.

Integrating our method with these other, complementary methods designed to analyse single-cell data is also important to identify which gene sets we wish to analyse. Datasets generated using RNA sequencing contain information for thousands of genes, but we recommend selecting at most several hundred to analyse — not only to allow inference in a reasonable timescale (Fig. 7) but also to aid interpretation of the resulting networks. Depending on our biological question and knowledge about the system we are studying, we can select genes using prior knowledge or functional annotations, or based on features such as degree of variability, differential expression between different cell types, or temporal variability in expression state; a number of single-cell specific methods have been developed to detect such features, e.g. (Bacher & Kendziorski 2016, Trapnell et al. 2014, Haghverdi et al. 2016, Reid & Wernisch 2016, Setty et al. 2016, Kharchenko et al. 2014, Korthauer et al. 2016, Vallejos 2016, Finak et al. 2015).

Technical noise and other biological sources of heterogeneity (e.g. transcription stochasticity, other cellular processes) within the cell population will, to some extent, impede our ability to detect statistical dependencies. We see that increased technical noise reduces our ability to recover the structure of the underlying network, although PIDC still achieves the best performance (Fig. S5C). The theoretical benefits of using information theoretical methods are clear for these data, as we expect the observed dependencies to be complex and non-linear under such scenarios. It is of course highly unrealistic to expect every cell to follow precisely the same route through transcriptional space when progressing through a biological process, e.g. differentiation. We do make the assumption however, that there are key changes in transcriptional state that must occur in order

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for cells to respond appropriately to environmental and developmental cues, and that these will be subject to conserved regulatory mechanisms. We aim to identify the strongest functional relationships between genes that occur across large parts of a given cell population, and infer network features that are common across disparate cells.

As with any statistical analysis, it is important to consider the limitations of our approach. Many of these are general to network inference approaches aiming to reconstruct GRN structure from mRNA profiles and have been discussed in depth elsewhere (Penfold & Wild 2011, Marbach et al. 2012, Villaverde & Banga 2013, Oates & Mukherjee 2012), but it is worth emphasising a few key points as these affect how we should interpret and use our results. Firstly, we can only hope to detect relationships where there is sufficient variability in gene expression observed under the chosen experimental conditions. Functional interactions will only be detectable if they induce changes in transcriptional state that persist over a reasonable timescale — we will not, for example, detect rapidly fluctuating changes, as the transient changes in mRNA levels will not result in observed statistical dependencies across a group of cells. As well as functional regulatory relationships, we are likely to also identify co-regulatory relationships where genes under the influence of the same regulatory mechanisms will show coordinated changes in expression. Without making further assumptions, or using perturbation or temporal data, we cannot distinguish the former *causal* relationships; however, in many instances it will still be biologically informative to understand which groups of genes respond in a coordinated manner. In cases where we believe the assumptions made by pseudotemporal ordering algorithms to be reasonable (Trapnell et al. 2014, Bendall et al. 2014, Haghverdi et al. 2016, Reid & Wernisch 2016, Setty et al. 2016, Moris et al. 2016), we could potentially make use of this temporal information to infer causality and directionality of gene interactions within networks (Villaverde et al. 2014, Villaverde et al. 2013, Zoppoli et al. 2010, Opgen-Rhein et al. 2007).

Methods that allow us to explore high-throughput datasets and identify putative functional relationships between genes are clearly needed to allow us to effectively analyse the large quantities of single cell transcriptomic data now available. Much of our existing knowledge of biological systems is gained from population-averaged bulk data, so although some inference algorithms aim to incorporate prior knowledge (Mukherjee et al. 2008, Studham et al. 2014), when working with single cell data we may want to avoid biasing our conclusions in order to fully exploit the potential of these data to provide new insights. The inferred relationships and network structure should be viewed as a way of identifying patterns and putative relationships from the data thus serving as a hypothesis-generation tool to guide promising areas for future research and investigation. Validating or invalidating any of these hypotheses experimentally may, of course, lead to a revision of the network model — like any mathematical model it should be subject to refinement as new experimental data becomes available. As with all network inference methods, we cannot expect to reconstruct the exact structure of the underlying biological networks, but instead view such methods as tools to explore the data; generate hypotheses; represent the current state of understanding; and guide further experiments, model development and analyses.

Methods

Discretization algorithms

In order to use the entropy estimators described here, continuous datasets must first be discretized. A number of algorithms exist to define the total number and boundaries of the resulting partitions (or bins). One common simple approach is to use bins of equal width, with the number of bins determined heuristically, e.g. here we use the nearest integer to the square root of the size of the dataset, $\sqrt{n}$ (Mosteller et al. 1977). A more sophisticated approach is the Bayesian Blocks algorithm (Scargle et al. 2013), in which the number and widths of bins are chosen by optimising a fitness function, without constraining the bins to be of equal width.

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Estimators

When a dataset is large enough, the empirical frequencies can be considered to be an approximation of the true probabilities, referred to here as the maximum likelihood approach. For sparser datasets, a number of methods have been developed either for estimating the probability distribution from a set of frequencies, such as the Dirichlet estimator and the shrinkage estimator, or for estimating the entropy directly, as in the Miller-Madow estimator (Hausser & Strimmer 2009, Paninski 2003).

The Dirichlet estimator refers to a group of Bayesian estimators that take a Dirichlet distribution as prior, but each with different parameters (Agresti et al. 2005, Hausser & Strimmer 2009). There is no consensus on the best parameters to use, despite several proposed alternatives (Hausser & Strimmer 2009); here, we use the same parameter, 1, for each bin unless otherwise stated.

The shrinkage estimator is also Bayesian, compromising between the observed frequencies, unbiased but with a high variance, and a prior (or target) distribution, biased but with low variance (Hausser & Strimmer 2009). The estimate is affected by both the choice of target distribution and the weight given to the target (or shrinkage intensity). In the current analysis the optimal shrinkage intensity is calculated as described in (Hausser & Strimmer 2009), and the target distribution is the uniform distribution.

The Miller-Madow estimator is an entropy bias correction that does not estimate the probability distribution, and therefore cannot be meaningfully applied to higher order information measures. Despite this it has been applied for the comparison of different MI-based algorithms (Meyer et al. 2008), and so it is included in this analysis, with the caveat that its meaning is unclear.

Simulation of *in silico* 3-gene network data

We considered six 3-gene topologies (Fig. S1), and used the Gillespie algorithm (Gillespie 1977) to generate stochastic simulations of gene expression timecourse data using two alternative model definitions (based on thermodynamic or mass action kinetics). In both cases, we include an additional activating stimulus that is present from halfway through the simulation timecourse. This additional stimulus acts to perturb the system away from a steady state, driving changes in gene expression that are necessary for relationships between genes to be observable. This section describes these model and simulation details.

The first *thermodynamic* model includes seven species: mRNA (x_i) and protein (y_i) corresponding to three genes ($i = 1, 2, 3$), and a stimulating ligand (s) which targets a selected gene. We define the following reaction types with associated propensities,

Reaction	Propensity
$x_i \rightarrow \emptyset$	λx_i
$y_i \rightarrow \emptyset$	λy_i
$x_i \rightarrow x_i + y_i$	$r_i(x_i)$
$\emptyset \rightarrow x_i$	$f_i(\mathbf{y}, s)$

to represent mRNA decay, protein decay, translation and transcription respectively, where λ is the protein / mRNA decay rate, translation is modelled according to saturation kinetics (with maximum rate $\alpha_{\text{translation}}$), i.e.,

$$r_i(x_i) = \alpha_{\text{translation}} \cdot \frac{1}{1 + (k_i/x_j)},$$

and transcription rates depend on the concentration of any regulating proteins (including the stimulating ligand s if present) according to the relationship,

$$f_i(\mathbf{y}, s) = \alpha_{\text{transcription}} \cdot \sum_{m=0}^{M} \alpha_m P\{S_m\},$$

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where $\alpha_{\text{transcription}}$ is a constant transcription rate, M is the total number of possible states S_m for gene i (either unbound, or bound by one or two regulating proteins), and α_m is the relative activation rate for each state. The probability of each state, $P\{S_m\}$, depends on the concentrations of the regulating proteins, modelled according to standard thermodynamic principles (see e.g. (Marbach et al. 2010) for details). For example, if a gene has two possible regulators (proteins y_j, y_k), we calculate the mean activation of transcription of the target gene i using the function,

$$f_i(\mathbf{y}) = \frac{\alpha_0 + \alpha_j\chi_j + \alpha_k\chi_k + \alpha_{jk}\chi_j\chi_k}{1 + \chi_j + \chi_k + \chi_j\chi_k},$$

where $\chi_j = (y_j/k_j)$, k_j is the dissociation constant, and the possible states of the gene are unbound, bound by y_j or y_k alone, or by both y_j and y_k. For our models, the maximum number of regulators for a given gene is three (proteins y_j and y_k plus the stimulating ligand s), but we assume a maximum of two regulators can bind the gene at any one time (so we consider the gene states with each possible pair of ligands bound, but not a state with all three bound).

For the thermodynamic model, we simulated timecourses from time 0 to 1000, with the stimulating ligand s present from time 500 at a constant level of 20 molecules, and recorded the system state at 41 equally spaced intervals. We repeated the simulations 25 times — and calculated PID scores using the resulting data (i.e. 1025 data points or 'cells' were used to calculate each PID measure)— with the stimulus targeting each of the three genes in the network in turn (75 simulations in total, 3 sets of PID scores). We randomly sample initial mRNA (x_i) and protein (y_i) levels from a $U(0, 5)$ distribution; we perform simulations for five different initial conditions and plot the mean PID profiles from these five different conditions in Figs. 2 and S1. Model parameters are $\lambda = 0.02, \alpha_{\text{transcription}} = 2, \alpha_{\text{translation}} = 2$, and $k_i = 50$ (for all $i = 1, 2, 3$). Relative activation rates for transcription, α_m, depended on the number of activating and inhibiting regulators present in each possible state S_m: $\alpha_m = 0.1$ (for the unbound state, i.e. basal transcription), 0.001 if an inhibitor was bound (we assumed inhibition dominated activation), and 5 if only activating regulators are bound.

The second *mass action* model that we consider also includes seven species: genes (g_i) and mRNA/protein (x_i) for $i = 1, 2, 3$, and the stimulating ligand s. We assume that protein and mRNA concentrations are equal (i.e. translation is instantaneous) and, unlike our first model, assume that a gene can only be bound by a single protein at any time. The possible reactions and associated propensities are,

Reaction			Propensity
g_i	$\rightarrow$	$g_i + x_i$	$k_{\text{txn}} \cdot x_i$
x_i	$\rightarrow$	$\emptyset$	$k_{\text{decay}} \cdot x_i$
$g_i + x_j$	$\rightarrow$	$g_i x_j$	$k_{\text{on}} \cdot g_i \cdot x_j$
$g_i x_j$	$\rightarrow$	$g_i + x_j$	$k_{\text{off}} \cdot g_i x_j$
$g_i x_j$	$\rightarrow$	$g_i x_j + x_i$	$k_{\text{regulated}} \cdot g_i x_j$

for basal transcription and protein decay, protein binding and unbinding from a target gene, and transcription from a gene bound to a regulating protein respectively (where $g_i x_j$ indicates gene i is bound by regulating protein j).

For the mass action model, we simulated timecourses from time 0 to 400, with the stimulating ligand present from time 200 at a constant level of 20 molecules, and recorded the system state at 21 equally spaced timepoints. We repeated the simulations 50 times with the stimulating ligand targeting each gene to give a total of 1050 data points ('cells') that are used to calculate PID measures. We initiated simulations with two copies of each gene ($g_i = 2$), no stimulating ligand, and initial mRNA/protein levels sampled from a uniform distribution ($x_i \sim U(0, 50)$); we perform simulations for five different initial conditions and calculate the mean PID profiles from these five conditions (plotted in Figs. 2 and S1). Model parameters are $k_{\text{txn}} = 1, k_{\text{decay}} = 0.05, k_{\text{on}} = 0.01, k_{\text{off}} = 0.25$, and $k_{\text{regulated}} = 10$ or 0.1 for activating and inhibiting regulation respectively. Information measures were calculated from these data using the Matlab package written by Timme *et al.* (Timme et al. 2014), following discretisation with the AstroML implementation of the Bayesian Blocks algorithm (Vanderplas et al. 2012, Scargle et al. 2013).

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Simulation of *in silico* GeneNetWeaver network data

Data are simulated using GeneNetWeaver (Schaffter et al. 2011), which was used in the DREAM5 (Dialogue on Reverse Engineering Assessment and Methods) network inference competition (Marbach et al. 2012). We compare the network inference algorithms using ten networks, five with 50 genes and five with 100 genes; for each network size there are two *E.coli* and three *S.cerevisiae* networks, with average node degrees ranging from 1.19 edges per node to 5.51 edges per node.

GeneNetWeaver uses dynamical models that consider mRNA transcription and translation processes and generates time series simulations using stochastic differential equations to model dynamical noise and a mixed normal and log-normal model to represent microarray noise. To mimic single-cell data, we simulated thousands of time series experiments for each network, using the default settings, with mRNA measurements generated according to the default settings and the default time points: times 0 to 1000, in steps of 50. We sampled a single time point from each time series, representing a single 'cell': for the large datasets, we sampled 100 cells from each of the time points (2100 cells in total); for the medium datasets we sampled 100 cells from time 0 onwards in time steps of 150 (700 cells); and for the small datasets, we sampled 20 cells from all time points between 0 and 300 (140 cells). Where the dataset size is unspecified, we have used the medium datasets. For our initial exploration of PID measures, we also used data simulated from a 10-gene network (shown in Fig. 3A); in this case we again used the temporal sampling scheme for medium datasets.

In order to test the robustness of our method to zero-inflated data, typical of single-cell experiments, we further simulated 'drop-out' datasets. Zero measurements appear to be a combination of technical errors and genuine lack of expression due to stochasticity or biological state, and are more common in transcripts with low abundance (Kharchenko et al. 2014, Brennecke et al. 2013). We simulated drop-out events at two rates, such that expression values in the lowest 50 % (high rate) or 20 % (low rate) for each gene had a 50 % probability of being recorded as 0.

Network inference performance metrics and comparisons

AUROC and AUPR curves are calculated by comparing the inferred networks (which assign a score to every potential network edge) to the 'true' network used to simulate data, and identifying the numbers of correctly (and incorrectly) assigned edges as the threshold for edge inclusion is varied. AUROC is calculated from the area under the ROC curve, which is a plot of the false positive rate (FPR) on the x-axis versus the true positive rate (TPR) on the y-axis. AUPR is the area under the curve for a plot of precision (y-axis) versus recall (equal to TPR) on the x-axis. These quantities are calculated as,

$$\begin{aligned} \text{TPR (or recall)} &= \frac{TP}{TP+FN} \\ \text{FPR} &= \frac{FP}{FP+TN} \\ \text{Precision} &= \frac{TP}{TP+FP}, \end{aligned} \tag{13}$$

where TP and FP indicate the numbers of true and false positives, and TN and FN are true and false negatives. For networks where the number of negatives is much greater than the number of positives, AUPR is considered a better metric for comparing algorithm performance (Murphy 2012, Davis et al. 2006).

We use these scores to compare performance of our method relative to existing inference algorithms (Figs. 5 and S5). We used the implementations in R package `minet` for the existing inference algorithms (Meyer et al. 2008); we used the default or suggested parameters within this package except for the tolerance, τ, for ARACNE for which we either used the default (for the results in Fig. S5) or 0.1 (for the results in Fig. 5, as recommended in the original publication describing this algorithm (Margolin et al. 2006a)) .

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Methods for analysis of real datasets

Published datasets

We analysed three published qRT-PCR datasets to illustrate our network inference algorithm. Normalised Ct values from Psaila *et al.* (Psaila et al. 2016) were subtracted from the assumed maximum, 40, and the resulting dCt values (for 87 genes and 681 cells) were used in our analyses. Normalised dCt values from Moignard *et al.* (Moignard et al. 2015) are used directly for our analyses; we used data from the 20 genes they represent in their network model and 3934 cells. Raw Ct data from Guo *et al.* (Guo et al. 2010) are treated as described by the original authors (dCt values are calculated assuming a limit of detection of 28, and normalised on a cell-wise basis by subtracting the mean expression of housekeeping genes *Actb* and *Gapdh*; all values corresponding to expression below the limit of detection are set to -15); we used data from 46 genes (i.e. we excluded the housekeeping genes used for normalisation) and 442 cells.

Availability of data and materials

The `InformationMeasures.jl` package is available from https://github.com/Tchanders/InformationMeasures.jl.

Competing interests

The authors declare that they have no competing interests.

Author's contributions

TEC, MPHS, and ACB designed and performed research, analysed data, and wrote the paper. All authors read and approved the final manuscript.

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Supplementary Information for Network inference from single-cell data using multivariate information measures

Thalia E Chan, Michael P H Stumpf, Ann C Babtie

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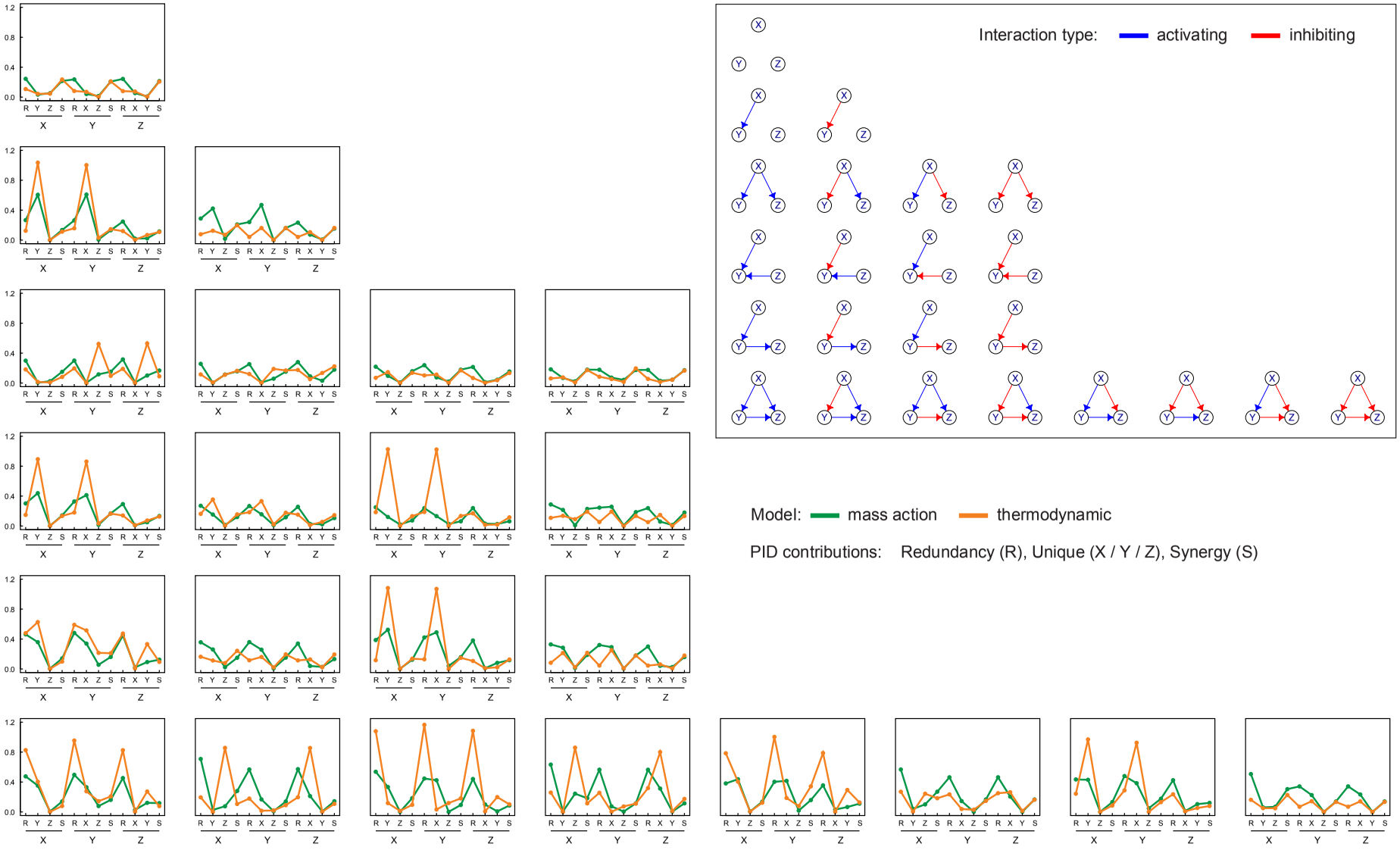

Figure S1: Related to Figure 2. Mean PID profiles for 3-gene network simulations, with stimulating ligand targeting gene X, and different network topologies. PID values were calculated using data simulated from 3-gene networks with the topologies illustrated in the top right inset (blue and red arrows indicate activating and inhibiting interactions respectively). Each line graph in the main figure shows the mean PID values calculated using models with the topology indicated in the equivalent grid position (i.e. the same row/column); the horizontal axis labels indicate the PID contribution, e.g. the first four values show the PID values with gene X as the target, consisting of the redundancy (R), unique contributions from gene Y (Y) and gene Z (Z), and the synergistic contribution (S). The models used for simulation assumed mass action (green) or thermodynamic (orange) kinetics, and the additional stimulation ligand targeted gene X from halfway through the simulation time (see *Methods*). The values plotted are the mean PID values calculated from five sets of simulations (with different randomly sampled initial conditions). Note that the same profiles are not necessarily seen for networks with equivalent connectivity, but different types of edges (results in the same row of this figure) — this is to be expected, as statistical relationships between genes can only be detected when there is sufficient variability in the observed data (which may not occur in some cases, e.g. if expression of a gene is inhibited throughout the simulation time).

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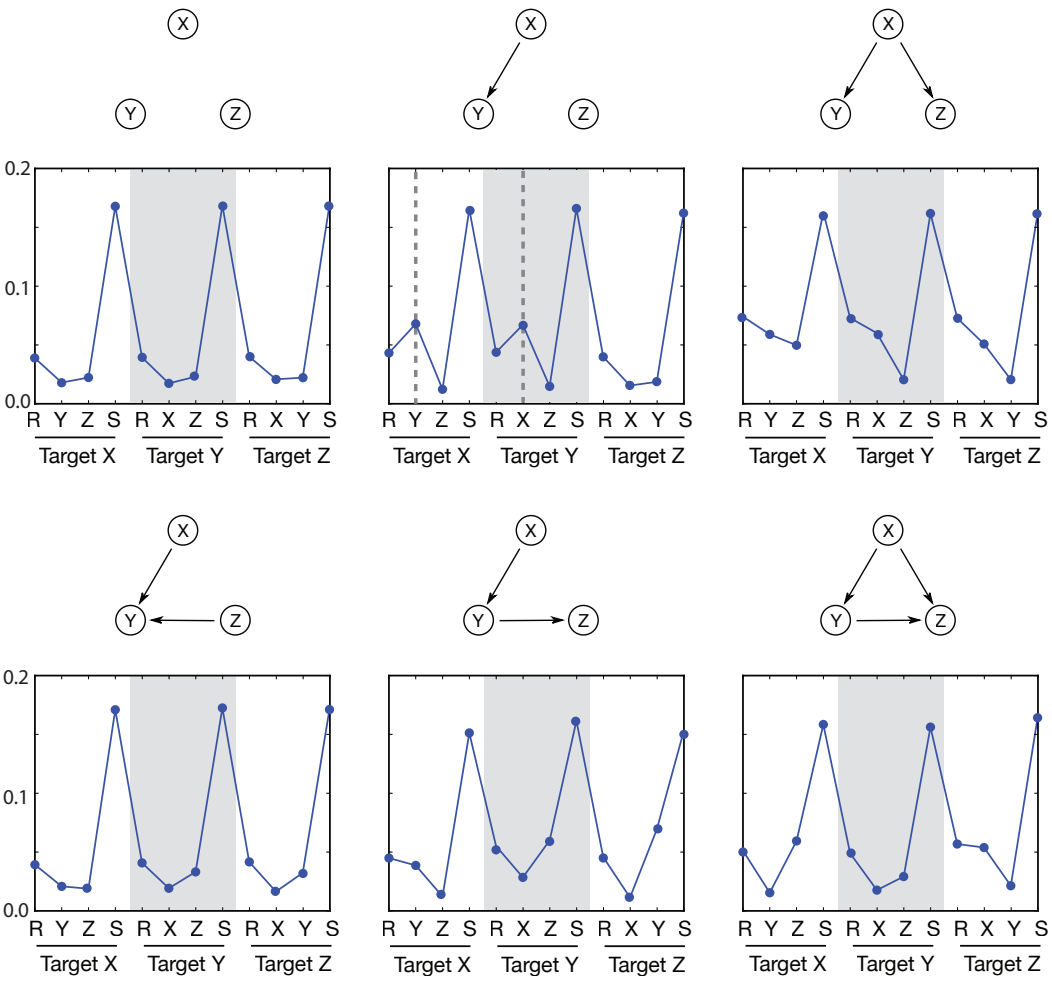

Figure S2: Related to Figure 2. Mean PID profiles for gene triplets in a 50-gene network. Every triplet of nodes (genes) in the network was assigned to one of six possible classes (based on the known connectivity of genes, as indicated in network diagrams above each plot). For each triplet, twelve PID values were calculated from the simulated data — there are four PID contributions with each gene treated as the target gene in turn, consisting of a redundant, synergistic and two unique contributions (Eq. 4). Each line graph shows the mean PID values calculated across triplets with the same topology, with the horizontal axis labels indicating the PID contribution, e.g. the first four values show the PID values with gene X as the target, consisting of the redundancy (R), unique contributions from gene Y (Y) and gene Z (Z), and the synergistic contribution (S).

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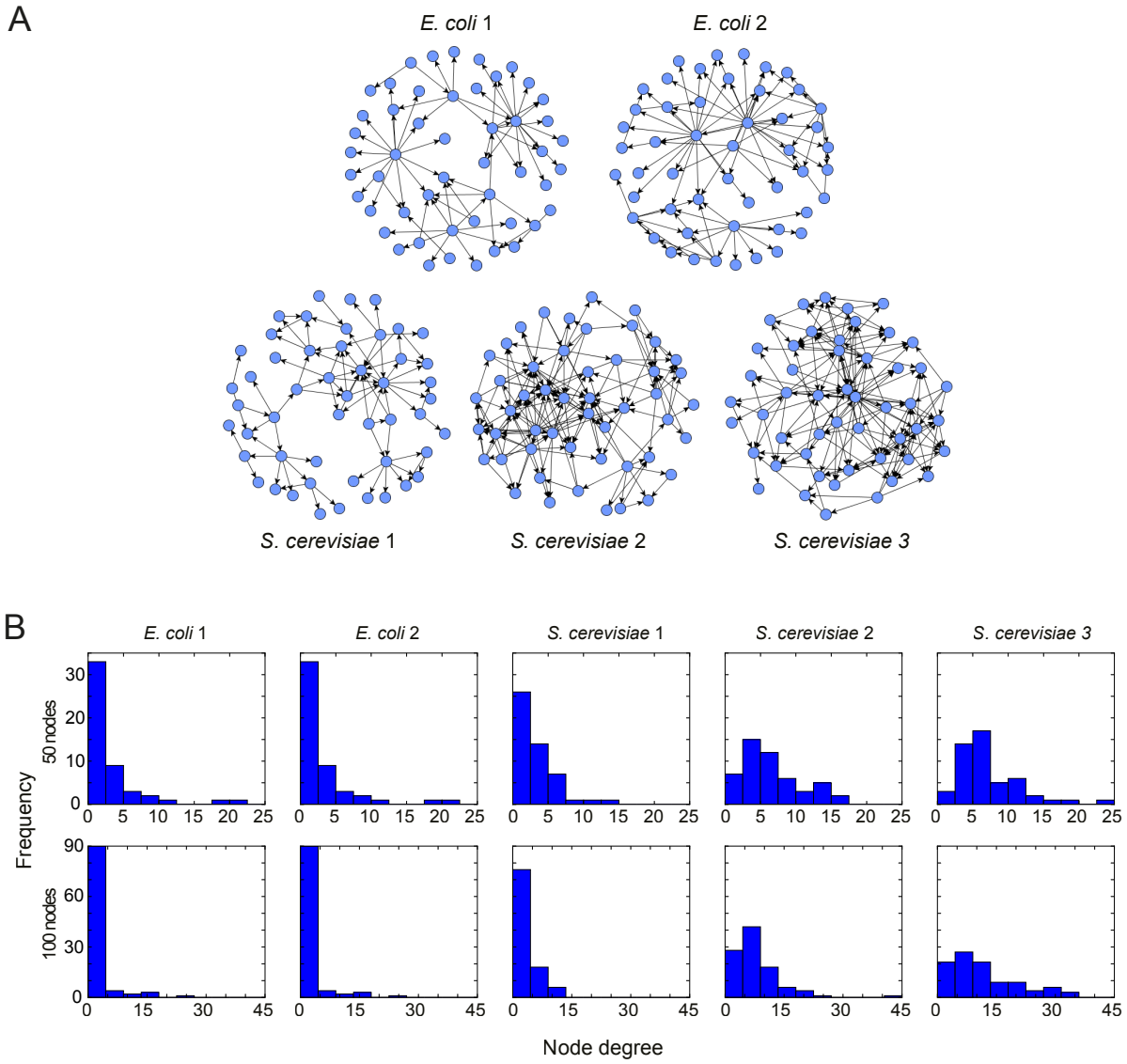

Figure S3: Related to Figure 3 and Table 3. Topological characteristics of the 50-node *in silico* networks. **A)** Visualisations of the directed networks, produced by GeneNetWeaver (Schaffter et al. 2011). **B)** Node degree distributions for each of the 50-node and 100-node networks. The distributions are varied, with the *E. coli* networks tending to have more hubs, and the *S. cerevisiae* networks tending to be more densely connected.

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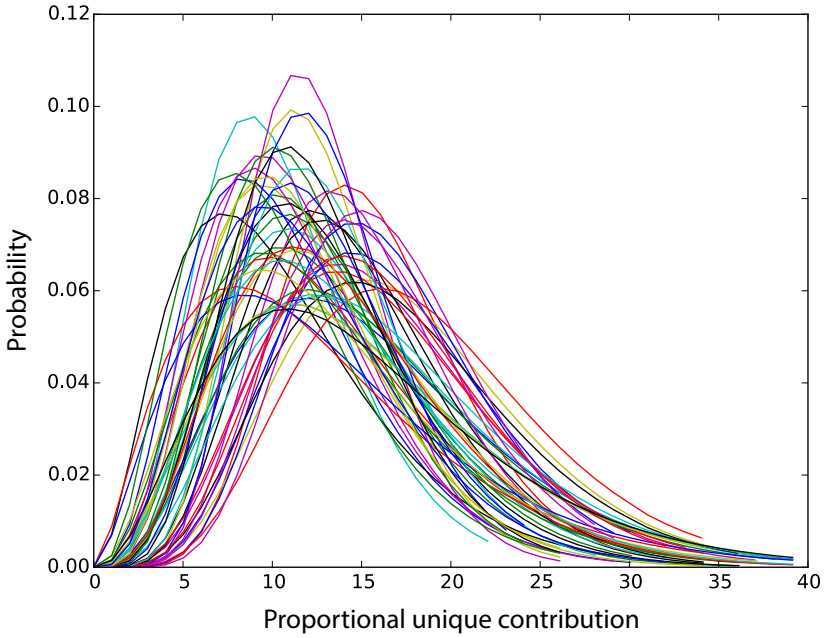

Figure S4: Related to Figure 4. Example empirical distributions of proportional unique contribution scores by gene. Gamma distributions were fitted to the proportional unique contribution (PUC) scores (Eq. 7) for each gene in a 50-node *in silico* network (for each gene X, a PUC score, $u_{X,Y}$, is obtained for that gene paired with each other gene Y in the network). Due to the variability of these distributions, using a universal threshold for inferring edges is problematic, thus we use the cumulative probability distributions for each gene to obtain a final confidence score for network edges.

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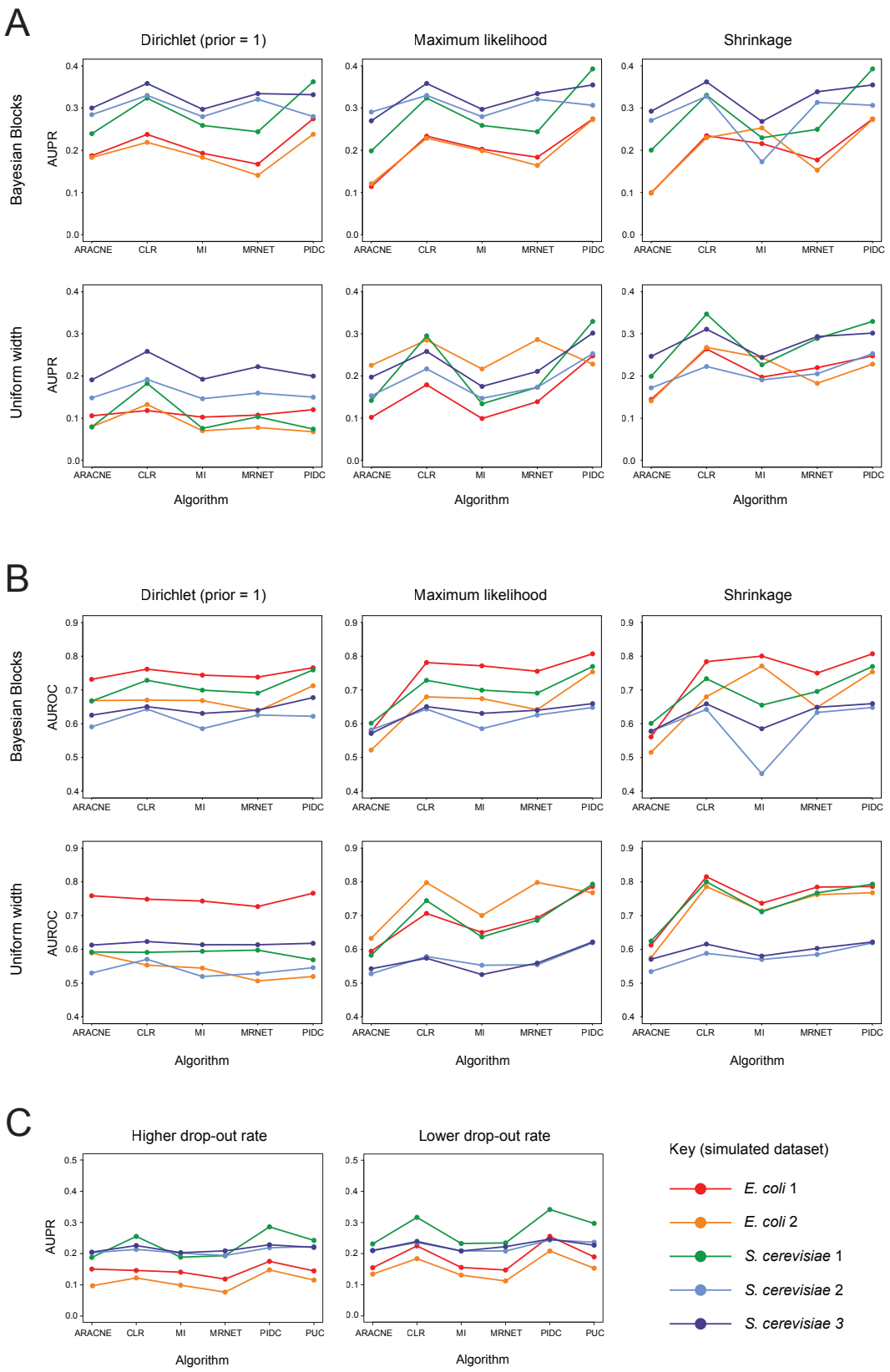

Figure S5: Related to Figure 5. Influence of discretization algorithm, estimator and technical error on network inference performance. AUPR (**A**) and AUROC (**B**) scores quantifying the accuracy of inferred networks calculated using *in silico* data simulated from five 50-gene networks (see *Methods*). Coloured lines indicate the results obtained with different datasets. Each plot shows the results obtained using a different combination of discretization algorithm (rows) and MI estimator (columns). Choice of algorithm and estimator clearly affects the relative scores of the network inference algorithms, suggesting that comparisons made using only one combination should be interpreted with caution; we find that the PIDC inference algorithm performs consistently well across the different combinations. **C)** From the medium-sized 100-node datasets, the lowest 20% (low rate) or 50% (high rate) of expression values for each gene each had a 50% probability of being set to 0; the recommended maximum likelihood and Bayesian blocks were used. The R package `minet` was used to implement the existing algorithms using the default parameters (Meyer et al. 2008).

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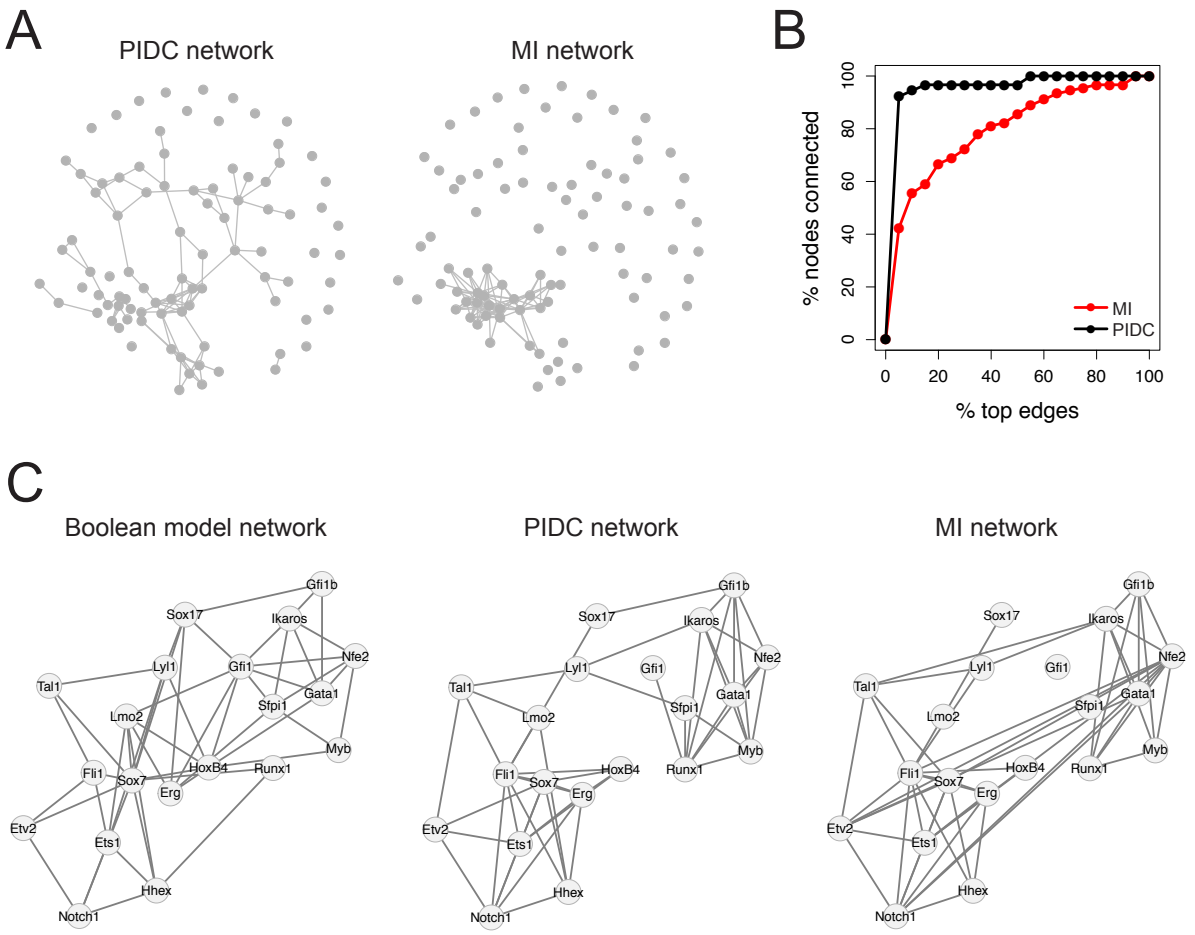

Figure S6: Related to Figure 6. Comparisons of networks inferred using MI and PIDC from experimental datasets. **A)** Comparison of the networks inferred from the data from Psaila *et al.* (Psaila et al. 2016) using our PIDC algorithm or MI relevance networks (in both cases, the top 2.5 % of edges are shown). **B)** Percentage of nodes that are connected in networks inferred from the Psaila *et al.* data (Psaila et al. 2016) as the threshold for edge inclusion is varied (from 0 to 100 % of possible edges, according to their rank). These results show that networks inferred using PIDC (black) tend to be better connected than networks inferred using MI (red); i.e. MI networks show more skewed degree distributions. **C)** Comparison of the networks inferred using haematopoietic development data in (Moignard et al. 2015) (as in Fig. 6D) showing the identity of nodes in these networks.

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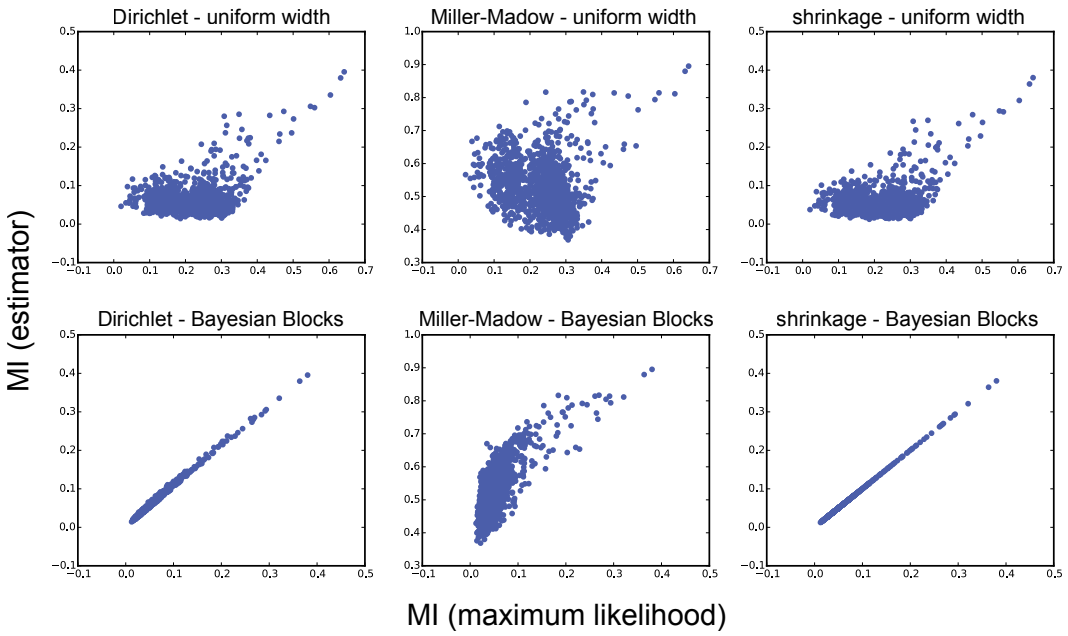

Figure S7: Related to Figure 5. Influence of discretization algorithm and estimator on MI rank. MI was estimated for every pair of genes in a 50-gene *in silico* network, using different combinations of discretization algorithms (uniform width or Bayesian Blocks (Vanderplas et al. 2012, Scargle et al. 2013)) and MI estimators (Dirichlet, Miller-Madow, shrinkage and maximum likelihood); see *Methods* for details. Each plot shows the relative ranks of MI scores obtained using the maximum likelihood estimator (horizontal axis) versus one of the other estimators (vertical axis); the top and bottom rows show results obtained using data discretized using a uniform width or Bayesian Blocks algorithm respectively. MI ranks were the most consistent when using Bayesian Blocks discretization (with the exception of the Miller-Madow estimator, which is an entropy bias correction that should not be used in higher-dimensional estimation and is included here due to its frequent misuse as an MI estimator).

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Table S1: Related to Figures 3 and S3. Topological characteristics of the 50-node and 100-node (in brackets) networks from which data were simulated by GeneNetWeaver (Schaffter et al. 2011). In all networks the vast majority of all possible node triples were either unconnected or had only one edge connecting two nodes in the triple.

Network	Nodes	Triples	Edges	Unconnected triples	One edge triples
E. coli 1	50 (100)	19600 (161700)	62 (125)	86.4% (92.8%)	12.0% (6.8%)
E. coli 2	50 (100)	19600 (161700)	82 (119)	82.8% (93.2%)	14.4% (6.3%)
S. cerevisiae 1	50 (100)	19600 (161700)	77 (166)	82.7% (90.3%)	15.9% (9.3%)
S. cerevisiae 2	50 (100)	19600 (161700)	160 (389)	66.6% (78.8%)	28.2% (18.9%)
S. cerevisiae 3	50 (100)	19600 (161700)	173 (551)	64.4% (71.6%)	29.7% (24.1%)

Table S2: Related to Figure S5 and Table S3. Joint entropy estimates, in bits, for up to four independent random variables, calculated using different estimators and uniform width discretization. Each variable is uniformly distributed over $n = 64$ bins and sampled 64^2 times, with theoretical entropy, $\log_2(n)$. The theoretical joint entropy of independent variables is the sum of their entropies. The Dirichlet estimator is given priors of $1/n$ or 1, and the shrinkage estimator is given a uniform target (as indicated in parentheses in table headings). The means of 100 repetitions are given, and the variances are all $< 10^{-4}$. Only the Dirichlet and shrinkage estimators produce accurate estimates with three and four variables.

Number of variables	Theoretical value	Maximum likelihood	Miller-Madow	Dirichlet $(1/n)$	Dirichlet (1)	Shrinkage (uniform)
1	6	5.9886	5.9963	5.9892	5.9998	6.0000
2	12	11.1705	11.4860	11.2234	11.8303	11.9998
3	18	11.9844	12.4804	15.9346	17.9915	17.9999
4	24	11.9997	12.4804	23.9283	23.9999	23.9998

Table S3: Related to Figure S5 and Table S2. Estimates of the difference between joint and marginal entropies of four independent random variables. The theoretical difference for independent variables is 0. Estimates are made for three sets of variables, drawn from three different distributions (uniform, normal or exponential). The Dirichlet estimator is given priors of $1/n$ and 1, and the shrinkage estimator is given a uniform target (as indicated in parentheses in table headings). The means of 100 repetitions are given, and the variances are all < 0.2. The estimators perform differently depending on the distribution: the Dirichlet and shrinkage estimator are the most accurate when given the correct prior, but can be the least accurate when the prior is wrong.

Distribution	Maximum likelihood	Miller-Madow	Dirichlet $(1/n)$	Dirichlet (1)	Shrinkage (uniform)
Uniform	11.9559	11.4868	0.0277	0.0428	0.0005
Normal	8.7172	8.2470	3.2126	3.1409	3.1389
Exponential	5.2646	4.8102	6.7636	6.5397	6.1207

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