Yambartsev et al. Biology Direct (2016) 11:52
DOI 10.1186/s13062-016-0155-0

Biology Direct

RESEARCH Open Access

Unexpected links reflect the noise in networks

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Abstract

Background: Gene covariation networks are commonly used to study biological processes. The inference of gene covariation networks from observational data can be challenging, especially considering the large number of players involved and the small number of biological replicates available for analysis.

Results: We propose a new statistical method for estimating the number of erroneous edges in reconstructed networks that strongly enhances commonly used inference approaches. This method is based on a special relationship between sign of correlation (positive/negative) and directionality (up/down) of gene regulation, and allows for the identification and removal of approximately half of all erroneous edges. Using the mathematical model of Bayesian networks and positive correlation inequalities we establish a mathematical foundation for our method. Analyzing existing biological datasets, we find a strong correlation between the results of our method and false discovery rate (FDR). Furthermore, simulation analysis demonstrates that our method provides a more accurate estimate of network error than FDR.

Conclusions: Thus, our study provides a new robust approach for improving reconstruction of covariation networks.

Reviewers: This article was reviewed by Eugene Koonin, Sergei Maslov, Daniel Yasumasa Takahashi.

Background

It is quite common, especially in biology, that in order to understand how systems transition from one state to another (e.g. from health to disease) scientists compare how parameters such as gene expressions, protein levels, or metabolite abundances differ between these states. One result of such a comparison is a list of parameters up- or down-regulated (due to the increase or decrease of some numerical value attributed to the parameter) from the first state to the second. In case of gene expression, these alterations represent a consequence of the two key factors: first, the original stimulus (e.g. mutation or environmental perturbation) that underlies the transition of a biological system from one state to another; and the second factor, a biological process that drives regulatory relations between individual genes independently on the presence of the stimulus. In other words, regulatory relations in biological systems (as well as many other systems) are not generally functions of the state but are rather pre-determined by biological roles of the components.

Most frequently, the components like genes are not regulated independently from each other; rather, they make up regulatory networks [1–5]. A common approach and the first step to the reconstruction of regulatory network structure is the inference of a correlation network built from parameters differentially abundant between two states. In particular, correlation (or, for the purposes of this paper, co-variation) networks are widely used in gene expression analysis.

Indeed, gene expression networks have been widely used to advance global understanding of principles that govern regulatory processes in biology [6, 7], to disclose molecular mechanisms of diseases [8], and even helping with finding better drugs [9]. Indeed, medical fields such as cardiology [8], endocrinology [10, 11], immunology [4, 12, 13], host-microbiome interactions [10, 12] and others have benefited from gene network analysis. Cancer is a very good example of applications of gene expression networks because

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insights from network analyses were essential for identification of key drivers of carcinogenesis for brain [14, 15], breast [15], skin [9] and cervical [16] tumors.

Co-variation network analysis works under the assumption that any edge (link) in a network, corresponding to a correlation between two parameters/nodes, is the empirical result of either direct or indirect (i.e. confounding) causal relationships, unless the edge is erroneously drawn (i.e. the observed correlation is an artifact of statistical error) [17–19]. Thus, we hypothesized that in a co-expression network there may be a relationship between the sign of correlation (i.e. positive or negative) of two regulated genes and the direction of their change between the two states (i.e. up or down-regulation). In this paper, we demonstrated the presence of this inter-dependence in different types of data, found that a departure from this relation reflects a proportion of erroneous edges in the regulatory networks, and developed a mathematical theory of this phenomenon.

Results

The concept of unexpected correlations

In order to verify whether there is a relationship between the direction of gene regulation and the sign of correlation we used a gene co-expression network from our recently published paper on network analysis in cervical cancer [16]. We felt that this network should provide excellent real data for this analysis, as it was constructed from a robust meta-analysis of five cancer gene expression datasets (GSE26342, GSE7410, GSE9750, GSE6791, GSE7803) and thus validated by large, independent sources. This network contained 738 nodes with 490 up and 248 down-regulated between cancer and normal tissues. These nodes were connected by 3161 edges with 2882 representing positive and 279 negative correlations. Relating these two types of information, we observed a strong association between the direction of gene expression change and the sign of correlation (Fig. 1a). Positively correlated genes in ~98 % cases had concordant increases or decreases in gene expression (up-up or down-down), and negatively correlated ones in ~92 % of cases were regulated in opposite directions (up-down). At first glance we found surprising such a strong association and sought to further evaluate this phenomenon. Thus we focused on a part of this big network, which is a bi-partite network consisting of 626 correlations between gene-regulators and gene-targets [16]. In this smaller network, in which correlation links could more obviously correspond to causal links (because gene-regulators have changed their expression as a result of chromosomal aberrations (Additional file 1: Figure S1)), we found similar association between direction of correlation and gene regulation (Fig. 1b).

We wondered whether such association can be generalized to other gene regulatory systems with two states (e.g. health and disease) and two types of regulation (stimulation and inhibition). In order to further investigate this, we propose a scheme in which we associate the sign of correlation (+/−) of each network edge with the direction (up/down) of gene regulation between system states. Sign association follows a simple set of rules:

- If there is a correlation between two “up” or “down” regulated genes (as in the top left panel in Fig. 1c), the sign associated with the link is positive.
- If there is a link between an “up” regulated gene and a “down” regulated gene (as in the bottom left panel in Fig. 1c), the sign associated with the link is negative.

The full set of possible combinations of gene regulations and correlations are given in (Fig. 1d). We hypothesize that correlations whose sign disagrees with the corresponding association are *erroneous*, i.e. they are the result of statistical error rather than causal relationships; or, they can be the results of an external/indirect influence, which is irrelevant for transitions between the biological system states. We will hereafter call such correlations *unexpected* (Fig. 1d), and their proportion among all correlations in a network is abbreviated as PUC (Proportion of Unexpected Correlations).

Since the original observation (Fig. 1) was made in complex system we also wanted to test the association between the sign of correlation and the direction of change in gene expression in the system where cause of gene regulation can be unambiguously defined. For this, we employed a basic principle claiming that a result of experimental perturbation represents a *bona fide* causality relationship. In the same cervical cancer work, we had performed siRNA perturbation of gene LAMP3 (GSE29009), which was one of the key gene-drivers of the antiviral subnetwork. Our theoretical prediction would be that genes whose expression is affected by perturbation of the gene-driver (i.e. LAMP3) *in vitro* and correlated to the expression of the gene-driver in the original cancer data should present correlations of the expected sign. For example, if a gene was down-regulated by LAMP3 siRNA, it is expected to be positively correlating to LAMP3 in the cancer gene expression data and vice versa (i.e. if gene is up after siRNA treatment correlation should be negative). Thus we analyzed if the direction of regulation of genes affected by LAMP3 siRNA in the cell line was corresponding to the sign of correlation between each gene and LAMP3 in four cervical cancer datasets (GSE7410, GSE9750, GSE6791, GSE7803). In these datasets, we observed that almost all correlations between LAMP3 and genes whose expression was affected by LAMP3 siRNA had correlation signs concordant to the directions of gene regulations due to siRNA treatment (Fig. 1e). Thus, this data provides the additional experimental support for our hypothesis about non-random

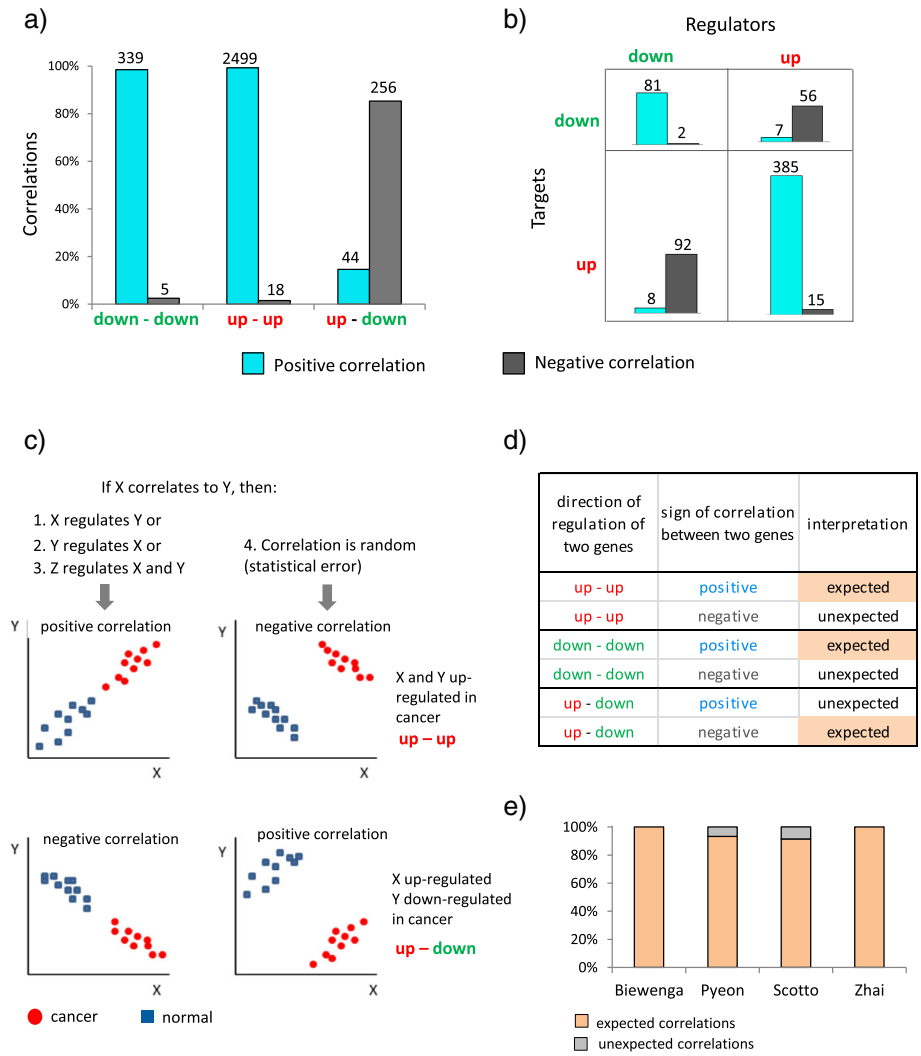

Fig. 1 Sign of correlations corresponds to the direction of change in regulatory networks. **a** Percentage of positive and negative correlations for pairs of up-regulated (*up*) and down-regulated (*down*) genes observed in the network from Mine et al., 2013; **b** number of positive and negative correlations between pairs of target and regulator genes in relation to their up- or down- regulation in cervical cancer data; **c** examples of regulatory (*left panels*) and erroneous (*right panels*) connections between genes X and Y; **d** possible combinations of gene regulations and correlations with the interpretation of connection; **e** percentage of expected and unexpected connections between LAMP3 and other differential expressed genes in cervical cancer corresponding to genes regulated after knockdown of LAMP3 in four datasets: Beiwenga (GSE7410), Pyeon (GSE6791), Zhai (GSE7803), Scotto (GDS3233)

interdependence between sign of correlation and direction of gene regulation.

Mathematical formalism

Encouraged by these results, to better understand the properties of this new metric (PUC) we went further to establish a rigorous mathematical framework.

Our hypothesis that unexpected correlations are erroneous can be rigorously proven for systems that transition between two stable states with two types of relationship between parameters: stimulation and inhibition. Herein, as an example, we provide a proof of our hypothesis using a simple Bayesian network[9] with two equilibrium states and linear dependences between nodes. The general case is considered in Section II.2 of the Additional file 1.

In order to formulate our results, we begin by stating the following mathematical notions and definitions. A regulatory network is represented as a directed acyclic graph (DAG) $G = (V, E)$. Any edge $e \in E$ is an ordered pair of vertices (nodes) $e = (v, w) \in V^2$. The order of vertices in an edge represents the direction of causality in a regulatory network (that is, in the edge (v, w), v regulates w). For any node v we associate the set of its parents as $pa(v) := \{u \in V : (u, v) \in E\}$. We define the set of root-nodes $gf(G)$ for the graph G as the set of all nodes

without parents: $gf(G) := \{v \in V : pa(v) = \varnothing\}$. For simplicity, we consider a regulatory network with only one root-node, $|gf(G)| = 1$, denoted by the vertex o. The case with more than one root-node is covered by the general model considered in Additional file 1, see SectionI.et graph G be weighted, meaning that every edge $e = (v, w) \in E$ has an associated label (weight) $c_{vw} \in \mathbb{R}$. With every node $v \in V$ we associate a random variable M_v. Variables M_v, $v \in V$, are connected by the following structural linear equations:

$$M_v = \sum_{w \in pa(v)} c_{wv} M_w + \varepsilon_v. \quad (1)$$

Here, the random variables ε_v, $v \in V$, representing the noise in the system, are mutually independent and identically distributed with mean 0 and variance σ_v^2. We suppose heteroscedasticity with uniformly bounded variances: there exists σ^2 such that $\sigma_v^2 \le \sigma^2$ for all $v \in V$. By defining the distribution of the root-node variable M_o, we obtain a unique joint distribution of random variables M_v, $v \in V$. This joint distribution will be referred to as *equilibrium state*.

In the previously discussed biological framework, a graph G represents the entire gene expression network. A node v represents a gene with the corresponding expression level M_v. . An edge $e = (v, w)$ represents a causal link between two genes v and w in which the expression of w is regulated by v, and c_{vw} is the interaction weight. The sign of c_{vw} reflects the direction of regulation: a negative (positive) sign corresponds to inhibition (stimulation). The parents of v are simply all genes which regulate v and the root-node of G is the primary regulator of the entire network.

In order to define two distinct equilibrium states, say P and Q (e.g. case and control, disease and health, etc.) for a system defined by causal graph G and structural equations (1), we need only to define two independent root-node variables, $M_o^{(P)}$ and $M_o^{(Q)}$, together with mutually indepennt noise variables $\varepsilon_v^{(P)}$, $\varepsilon_v^{(Q)}$, $v \in V$. Let $M_v^{(P)}$ and $M_v^{(Q)}$ denote the expressions of the gene at node v in two distinct equilibrium states P and Q. For any v we denote the changes in expression between states as $\Delta_v = \mathbb{E}(M_{v^{(P)}}) - \mathbb{E}(M_{v^{(Q)}})$, where $\mathbb{E}$ denotes the expectation value (mean) of corresponding variable.

The mathematical definition of expected and unexpected links, as introduced informally in the introduction, is now formally expressed in the following definition.

Definition. *An edge $e \in E$ is called an expected link between nodes $v, w \in V$ if and only if $\Delta_v \Delta_w cov(M_v^{(P)}, M_w^{(P)}) > 0$ and $\Delta_v \Delta_w cov(M_v^{(Q)}, M_w^{(Q)}) > 0$. An edge which is not an expected link is said to be an unexpected link.*

This definition effectively states that the directions of regulation of two genes between two states should agree with the sign of the correlation between them within each state.

Note that the covariances in the definition can be substituted by the coefficient of correlation (Pearson correlation).

In the main lemma stated below, we show that here, all unexpected links are produced by the noise in the system: i.e. if σ^2 is small enough, then the system will have no unexpected links.

Lemma 1. For any finite DAG with linear structural equations (1) and two equilibrium states there exists some σ_0 such that if $\sigma_v^2 < \sigma_0^2$ for all $v \in V$, then there are no unexpected links in the system.

The proof is given in Section II.1 of the Additional file 1. Another very important property of the concept of unexpected links is that PUC represents and identifies approximately half of all erroneous correlations:

$$2\mathbb{E}(PUC) \approx \mathbb{E}(\text{total proportion of false positive links}) \quad (2).$$

A formal proof of this statement (under certain conditions) is given in Section III.3 of the Additional file 1, as well as an explanation for why this makes intuitive sense. The basic idea is that false edges are, in principle, equally likely to have expected correlations as they are to have unexpected correlations.

Unexpected correlations reflect the noise in real and simulated networks

Lemma 1 shows that in regulatory networks unexpected correlations must have appeared as a result of noise within the network and that the proportion of unexpected correlation thus reflects the noise level in a network.

Mathematical models are restricted by the domain of their assumptions, which limits their applicability. Thus, although we have empirically observed a small PUC in a high confidence cervical cancer network (Fig. 1a-b), we wanted to verify whether this correspondence would still hold in different settings. We therefore analyzed 24 additional data sets retrieved from the BRB Array Tools Archive (see Additional file 1) providing gene expression network transitions in different types of cancer, and found that PUC strongly correlated with FDR (correlation coefficient of 0.87 CI95% 0.8117–0.9416). Turning our attention to phenomena other than cancer, we also analyzed the gene expression network perturbed as a result of colonization of intestinal tissue with normal microbiota (i.e. the mix of microorganisms that live in the gut). In these data (GSE60568) [13] and again found that PUC is highly correlated with FDR (Fig. 2f).

Thus, the observation of strong correlation between FDR and PUC in multiple datasets from diverse

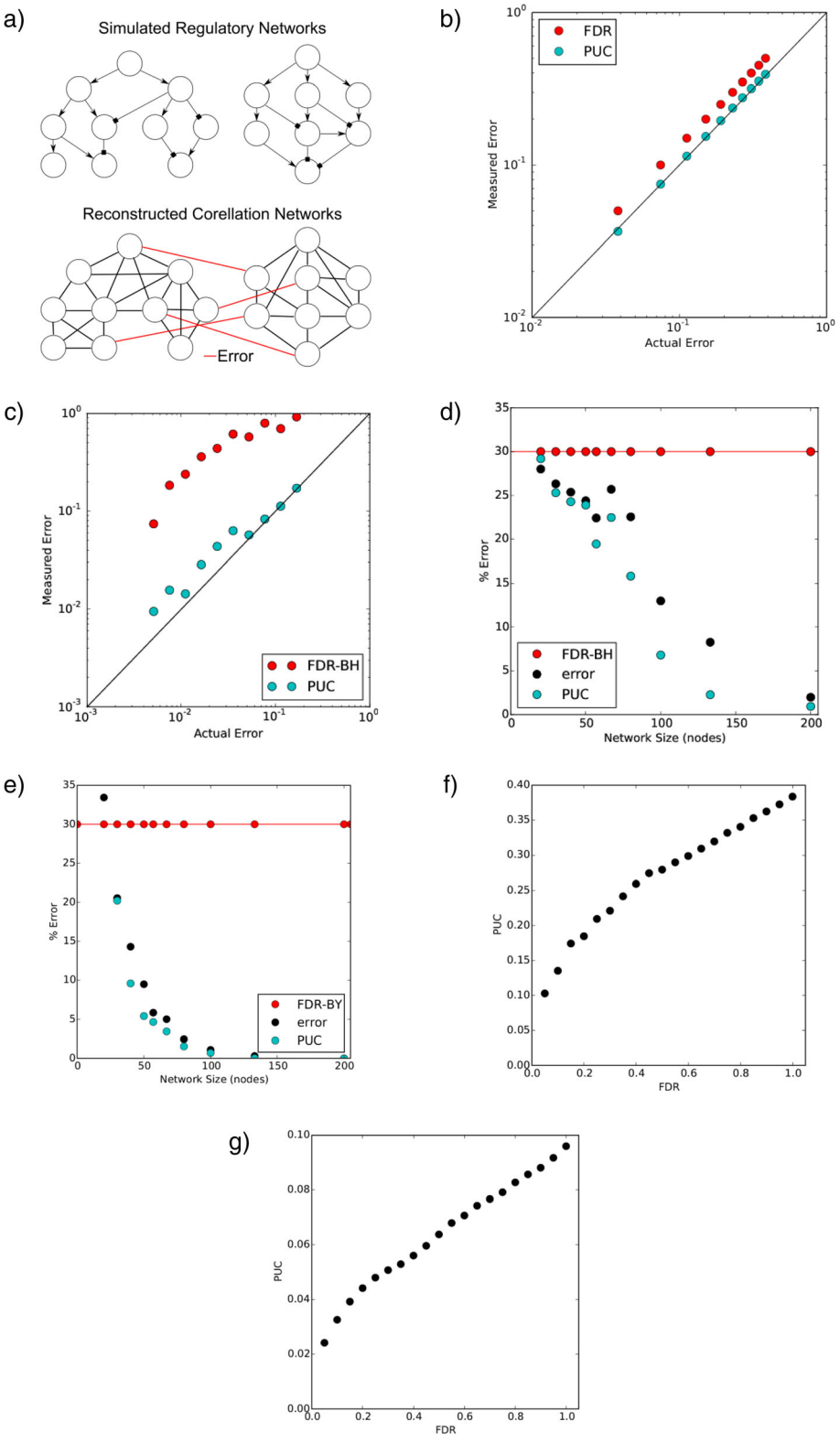

Fig. 2 (See legend on next page.)

(See figure on previous page.)
Fig. 2 Comparison of PUC and FDR. **a** Two regulatory networks are simulated independently, then both networks' node expression levels combined into one data set. In a correlation network constructed from the simulated data, any correlations (links) between nodes from independent networks are known to be erroneous; Bayesian simulations (**b**), as well as gene regulatory simulations performed GeneNetWeaver (**c**) suggest that PUC more accurately reflects network error than FDR (Benjamini-Hochberg, FDR-BH); as network size grows, PUC more accurately reflects network error than FDR-BH (**d**) or its variation with multiple hypothesis under dependence called FDR Benjamini-Yekutieli (FDR-BY) (**e**); PUC correlates with FDR in both gene expression (**f**) and macroeconomic (**g**) data

biological settings in two different species (*Homo sapiens* and *Mus musculus*) provides additional support for our prediction that PUC, similarly to FDR, quantitatively reflects network error.

An important question, however, is whether PUC brings any advantage over the standard approach to measuring the proportion of erroneous edges in a reconstructed regulation network (i.e. FDR). Real data makes such a comparison difficult, because though both methods of analysis will return values for network error, there is not necessarily any obvious way to determine which is more accurate; i.e. in real data, the actual regulatory network is not known.

To investigate the behavior of PUC in a "controlled environment" we simulated networks using two approaches. We have used simple Bayesian networks [20] with structural equations (1) and the software GeneNetWeaver [21] which uses ordinary and stochastic differential equations as models for gene regulation. To compare the effectiveness of PUC and FDR, we construct a regulatory network as two distinct disjoint regulatory sub-networks, and gene expressions are simulated independently according two equilibrium states. In an empirical correlation network constructed from the simulated data, any correlations (link) between nodes from distinct sub-networks are known to be erroneous (Fig. 2a). This design allows for a true measure of network error against which to compare PUC and FDR analysis results.

In order to determine which method (FDR or PUC) better quantifies error, we look at all three measures of error (FDR, PUC, and the true error) and compare the accuracies of FDR and PUC. The results of both types of simulations suggest that PUC is more accurate than FDR in estimating true error, although there is a strong correlation between the two metrics (Fig. 2b,c).

The FDR family of methods is the most popular procedure for large-scale p-value correction for multiple hypotheses [22–26]. All these FDR methods, however, ignore the dependence structure between hypotheses, which leads to the fact that FDR is an overly conservative approach (i.e. it overestimates the number of false positives).

In the case of regulatory networks, each edge constitutes a hypothesis; interdependency of regulatory network hypotheses manifests in indirect regulation between genes. Indeed, this is exactly the case with co-variation networks, in which it is possible to find numerous indirect pathways with only a few direct links.

Using PUC as a measure of error, however, does not require any assumption about independence of hypotheses. PUC may thus be more accurate than FDR for error estimation in co-variation networks with a large number of interconnected nodes. The "degree of dependency" between hypotheses also depends on the size and number of sub-networks that compose a network. A network made up of twenty sub-networks consisting of twenty nodes each should have a lower degree of hypothesis interdependency than a single network consisting of four hundred nodes lacking any well-defined sub-networks.

In order to pinpoint this effect we simulated various networks up to 400 nodes in disjoint sub-networks, each with an equal number of nodes (for example, 20 disjoint sub-networks with 20 nodes each). While both types of simulations (Bayesian and GeneNetWeaver networks) showed overall more accurate results for PUC, in Bayesian networks we also observed lower efficiency of FDR for large networks (Fig. 2d). This effect, however, was less pronounced in GeneNetWeaver- simulated networks (Additional file 1: Figure S4). Furthermore, we obtained similar results (Fig. 2e) by using another version of FDR which was designed to correct for hypothesis interdependency – Benjamini-Yekutieli, FDR-BY [27].

PUC in a non-biological system

The fact that we could mathematically prove the relationship between unexpected correlations and network error suggests that this principle could be widespread beyond gene interactions in biological systems. As a proof-of-concept of PUC's generality, we turned our attention to economics. The justification for this choice of subject relates to the presumption that economic systems, similarly to biological systems, are governed by cause-effect relationships and can, by extension, be described by regulatory networks. We analyzed 1503 parameters retrieved from World Bank economic databases for the year 2008 in 193 countries in such areas as business, education, health, etc. (details provided in the Additional file 1). Parameters with bimodal distributions defined distinct states of economic networks for any given country. Figure 2f shows PUC for parameter

correlation networks with different FDR thresholds in which a particular parameter (expenditure per student on primary education as a percent of GDP per capita) defined distinct network states; Figure S2 (in the Additional file 1) shows similar graphs for various other parameters. As expected, these networks demonstrated a high concordance between the network errors given by PUC and FDR. This result supports the idea that the concept of unexpected correlations can be extended to a large variety of causal networks and that measurement of the proportion of unexpected correlations (PUC) can improve network analysis in a variety of scientific disciplines.

Estimating error using PUC

The entire procedure of PUC for calculating network error is as such: first, all correlations in a differential expression list are ranked by p-value. A network is constructed with edges consisting of correlations within an arbitrary p-value threshold (e.g. 0.01). Unexpected links are identified, counted, and removed from the network. The final measure of error in the remaining network is given by, where and are respectively the numbers of total and unexpected links in the network prior to removal of unexpected links. The reason for this formula is explained in the last paragraph of mathematical formalism section, and has to do with the fact that the number of unexpected links in a network is approximately equal to half of the total number of false links.

Discussion

The growth of molecular biology has advanced such that we can measure the expression of thousands of genes simultaneously. Simply measuring the expression of multiple individual genes, however, is insufficient to describe a systems issue such as complex diseases. To relate gene expression to physiological states (e.g. disease) and other variables in an organism's environment we utilize gene expression networks. These networks enable more intelligent identification of molecular subtypes of diseases and molecular targets for treatment. The reconstruction of gene expression networks, however, is not easily accomplished. Constructing reliable gene expression networks with current methods requires obtaining large data sets because a large number of hypotheses are required to be tested for network inference.

Although the False Discovery Rate (FDR - Benjamini-Hochberg) is the most popular multiple hypothesis correction method, its application for network inference is a conservative procedure and makes the often unfitting assumption of the independence between correlations in gene networks. There are less popular versions of FDR, for example Benjamini-Yekutieli [27], which take into account various dependence structures between the hypotheses under consideration, but the usage of this did not demonstrate any significant advantage over PUC (see Fig. 2e). Consequently, these corrections tend to have a high rate of false negative discovery (i.e. low power) and require vast sample sizes in order attain desirable degrees of certainty about reconstructed networks. There is thus a critical need for more powerful methods of estimation of false positive connections between genes in co-expression networks.

In this study we have revealed and mathematically proved a new feature of co-expression networks. This feature is based on the natural notion that any correlation has direct or indirect causal components and noise components. In the case when causal components prevail over noise, the sign of a correlation between two genes should be related to their up- or down- regulation of the genes between two states (Fig. 1). We first observed this relation empirically in gene expression datasets [16, 28], and subsequently in macroeconomic data (see Fig. 2f and Additional file 1: Figure S2). The observation of this network feature (relation between sign of correlation and direction of change) in data of such a different nature (biology and economics) suggests that this relation is a universal property of covariation networks.

We proposed using this relation for identifying false connections in co-variation networks increases network accuracy and estimates network error. This approach demonstrates clear advantage over the classic method (FDR) not only by providing better estimates of error in large co-variation networks, but also by allowing the removal of approximately half of all erroneous edges.

The identification of unexpected correlations has two primary impacts. Firstly, it provides a new method to estimate the proportion of erroneous links in a network. Secondly, it allows for the *removal* of approximately half of the erroneous edges in the network (namely, those that are unexpected), decreasing their proportion by a factor of two and thereby improving the overall accuracy of the reconstructed network.

The concept of expected and unexpected correlations that we introduced is closely related to the concept of monotone causal effects and the covariance between them [29]. Where the authors proved (see the theorem 4 in the paper [29]) that the covariation between any two positively (negatively) monotonically associated variables is non-negative (non-positive). It corresponds to our definition of expected correlations. Lemma 1 we proved for linear relations should therefore hold for any monotone relationships; this idea is expanded in Section II.2. of the Additional file 1, and the framework of PUC extended to a broader class of networks than those mentioned thus far.

It is also important to further investigate how non-monotonicity affects the notion and application of

unexpected correlations. The concept of non-monotonicity can be exemplified for our problem as different types of relationships in two network states, such as a negative correlation between parameters in one biological state and a positive correlation in another. In such cases, despite violation of monotonicity, we expect unexpected correlations to arise primarily due to noise, rather than the change in relationships. Nonetheless, we demonstrated (see Section II.4. of the Additional file 1) that there is no evidence for non-monotonicity to suggest that these exceptionally rare non-erroneous correlations are in fact responsible for the observed changes in gene expression between states of a biological system. Therefore, because the ultimate goal of network inference is actually to model and understand the transition of biological system from one state to another, we can safely remove these unexpected correlations from the reconstructed network for independent reasons (i.e. that they do not have causal contribution to system state transition).

We believe that this work, besides revealing a new feature of co-variation networks, introduces an entirely new way of dealing with error in their reconstruction. Indeed, statistical methods employed for such problems normally estimate an error, but cannot detect erroneous edges. We propose a method that besides (according to simulations, potentially superior) error estimation allows for identification and removal of approximately half of total network error. Thus, the identification and removal of unexpected correlations decreases the proportion of irrelevant and erroneous connections and strongly increases the power of network inferences.

Conclusion

This study reports a discovery of a new property of interdependence between sign of correlation and direction of gene regulation for covariation networks first observed by us in cervical cancer. It appears to be universal as it has been further found in wide range of phenomena within biology and economics. Furthermore, the newly revealed property provides a basis for developing a method for measuring the proportion of erroneous edges in a network. This method stands out among standard approaches like the false discovery rate (FDR), because besides estimating an error it allows for the elimination of about half of all incorrect links in a network under a given statistical threshold.

Reviewers' comments

Reviewer's report 1: Eugene Koonin, NIH

Reviewer comments:

In this paper, Yambartsev and colleagues develop a new approach to the analysis of covariation networks that is specifically applied to networks of gene co-expression from cancers and normal tissue controls. They make a straightforward observation that to me is quite intuitive, namely that there is a link between the sign of correlation between between expression profiles of a pair of genes and the directionality of their regulation between the compared states. In other words, if in a pair of genes, both are either down-regulated or up-regulated in cancer compared to normal tissue, they are expected to show a positive correlation. Conversely, if the directions of the regulation in a pair of genes, are different, they will show a negative correlation. The authors demonstrate the validity of this connection on experimentally characterized examples and simulated networks and prove analytically that such a connection should exist. They then employ this link to introduce a very simple but apparently powerful metric for measuring noise in covariation networks, namely PUC (proportion of unexpected connections), i.e. the fraction of edges in a network that violate the above rule. Remarkably, the PUC appears to perform significantly better than FDR. As far as I can see, the approach developed in this work can become important in the analysis of covariation networks, especially in the context of the comparison between different states (disease vs normal, normal vs stressed etc.) which is becoming increasingly important.

Reviewer question/comment and authors' response:

- The two most important ones seem to be the description of the comparison of PUC vs FDR and its statistical significance and the description of the networks and correlations themselves.
  Authors' response: *We agree that this is a very relevant question that will be answered in our future studies. However, in our simulation results we see so striking differences that statistical significance is obvious (Fig. 2b,c). Furthermore, despite we performed two types simulations (that are considered current standards in the field) it is not clear to what extent these results can be extrapolated to real biological systems. Therefore, we started a new investigation that attempts to disclose which properties of biological system are required for PUC to outperform FDR. As suggested by reviewer, this investigation actually involves statistical comparison between FDR and PUC.*
- Although less critical, I think it is highly desirable to expand the Background section to provide an adequate background on network analysis for cancer and other disease states.
  Authors' response: *We added the text in the introduction devoted to usage of gene expression networks in biology and biomedical research.*

Reviewer's report 2: Sergei Maslov, University of Illinois at Urbana-Champaign

Reviewer comments:

The manuscript describes a statistical method for filtering spurious edges in co-expression networks and thus estimating and improving the overall quality of the network. The main idea of the method is simple and seems to be correct as long as expression samples could be subdivided into two principal subgroups capturing the vast majority of expression variability (e.g. cancerous vs. normal tissues in the example used in the manuscript). In this case one expects the majority of positively co-expressed edges to connect genes that both went up or down in cancer-vs-normal comparison, while negatively co-expressed edges to connect genes that change in the opposite directions. This is true as long as the system has only two main attractor states. What is missing from the manuscript in my opinion is the rigorous discussion of conditions when this two-state model holds and when it does not. How does one separate spurious edges caused by statistical fluctuations from bona fide biologically meaningful gene- gene interactions in attractor states other than the one considered important by the authors of the expression collection (e.g. Comparison of gene expression in cancer vs. normal tissues)? In other words, the Eq. 1 indicates that the method focuses on the eigenvector corresponding to a single (the largest?) eigenvalue of the correlation matrix c_wv, while classifying other potentially biologically meaningful eigenvalues and eigenvectors as noise. I think that authors should spend more time discussing this major limitation of their approach.

Reviewer question/comment and authors' response:

- What is missing from the manuscript in my opinion is the rigorous discussion of conditions when this two-state model holds and when it does not.
  Authors' response: *Thanks for the question. We made modifications to the text to clarify the notion of equilibrium states in our paper.*
  In the paper we model a system as a Bayesian probabilistic network on directed acyclic graph (which represents causal relation between parameters) and with fixed structural equations. The joint distribution of parameters (i.e. equilibrium state) is defined by the distribution of noise variables (it is a product of distributions with bounded variances) and the distribution corresponding to causal root-nodes. Thus, having two distinct distributions on root-node, we will have two equilibrium states of a system.
  In the section "Mathematical formalism" we introduced the notion of equilibrium state more explicitly. We are sure that it is possible to generalize the notion of expected and unexpected links for the case of multi-state systems, but our scope here is only two-state systems. The two-states systems are very popular and commonly accepted in biology for example case–control studies.
  The notion of "equilibrium state" can refer also (as the notion of attractor) to some (stochastic) process in time. But it is possible to interpret an equilibrium state as an invariant distribution of this stochastic process. Changing some parameters of process we can change an invariant distribution, obtaining two or more equilibrium states. Moreover, we can deal with invariant distribution (equilibrium state) without considering directly an underlying process.
- How does one separate spurious edges caused by statistical fluctuations from bona fide biologically meaningful gene- gene interactions in attractor states other than the one considered important by the authors of the expression collection (e.g. Comparison of gene expression in cancer vs. normal tissues)?
  Authors' response: *Lemma 1 showed that in simple mathematical models (Bayesian networks defined on DAG with one root-node with monotone structural relations) all unexpected links are generated by a noise. The same is true for more general mathematical models, when the distribution of parameters satisfy some monotone relations. In practice, in rare occasions the unexpected correlations may appear as a result of true biological gene-gene interactions. However, according to Lemma 4 (see Section II.4 in Additional file* 1*) those rare interactions would not contribute to changes in gene expression observed between two states.*
- In other words, the Eq. 1 indicates that the method focuses on the eigenvector corresponding to a single (the largest?) eigenvalue of the correlation matrix c_wv, while classifying other potentially biologically meaningful eigenvalues and eigenvectors as noise. I think that authors should spend more time discussing this major limitation of their approach.
  Authors' response: *We would like to study possible extensions of our work, and really we want to discuss more about limitations of the approach. We plan to study how the structural equation can contribute in the existence of unexpected links for example through eigenvector of corresponding matrix of structural equations. But we wanted to fix our first step with the simplest cases considered in the paper.*
- A minor comment: on line 46 of the same page as Eq. 1 authors use EM as opposed to a more traditional notation E(M) to denote the expectation value of M. Both notations are potentially confusing as just a few lines below E denotes the set of edges

in the network. I recommend switching to or M_bar to denote the mean value.

Authors' response: *We thank the reviewer and we fixed the notations of expectation using suggested traditional ones.*

Reviewer's report 3: Daniel Yasumasa Takahashi, Princeton University

Reviewer comments:

This is a well written and very creative work that I enjoyed reading. The authors introduce a new concept called expected/unexpected link to infer which links on a network can be the result of noise (i.e., independent of the phenomena of interest). The idea is quite simple and elegant: If the relationship between the nodes is monotonic, the sign of the changes of the values associated to pairs of nodes in two different conditions should be the same. The assumption of monotonicity is quite general, at least comparing to most of the assumption in any network analysis of biological data, e.g., linear relationship. Therefore, the proposed method is expected to be quite useful and general. The application on cervical cancer network that motivates the article is very convincing. The idea of using siRNA experiment to validate the findings is excellent. The mathematical model and the proof of the claims seem to be correct to best of my understanding. The comparison with FDR is quite striking and this section alone should be enough to motivate people to look into the proposed method (myself included). The proposed method has the potential to become a landmark tool in network analysis.

Reviewer question/comment and authors' response:

I have only minor comments:

1) On Fig. 1e, it would be insightful to also show the proportion of expected/unexpected correlations between LAMP3 and the genes whose expression was NOT affected by LAMP3 siRNA. Given that the article is about false positive control and not so much about false negative control, the figure I am proposing could be in the Additional file 1.

   Authors' response: *We understand the interest reviewer to evaluate what happens with genes not affected by siRNA treatment. The problem with the request to "*show the proportion of expected/unexpected correlations between LAMP3 and the genes whose expression was NOT affected by LAMP3 siRNA*" is that in order to define correlations as expected or unexpected they have to be regulated. Therefore, if there is no regulation of given gene (by siRNA) we cannot classify correlation between this gene and LAMP3 in any category.*

2) The title for the subsection "Mathematical formalism relating causation and the sign of correlation" is misleading as most people would associate causation to direct links in a network. Probably something like "Mathematical formalism relating causal propagation and the sign of correlation" should be more adequate, as the authors use the word "causal propagation" later in the proofs.

   Authors' response: *We agree about misleading and we decided to change the title for simplest "Mathematical formalism".*

3) In the section "Mathematical formalism relating causation and the sign of correlation", I recommend the authors to try to give concrete and biologically motivated examples for the concepts that they introduce. That will make this section more readable and interesting. For example, what is the meaning of a weight Cvw? What is the meaning of two equilibrium states? The authors can say explicitly that in their cervical cancer example, states P and Q represent normal and cancer, for example. The authors also can say that the assumption of equilibrium states implies that the effect of perturbation in some subnetworks had time to propagate to the entire network. In eq (1) what does mean that the variance is small?

   Authors' response: *We revised this section adding interpretation of the weights as force of interactions between genes and adding the definition of the notion of equilibriums state as a joint distribution of parameters (gene expressions). We hope that in revised version it is more clear that the perturbation variables in structural equations (1) correspond to the internal noise in the system. The lemma says that if the internal noise is small enough (i.e. the variance of the perturbed terms is small) then there are no unexpected links in the system, it means, in our interpretation, that the unexpected links appear in these systems as a result of a noise.*

4) The authors should discuss in more detail the work by VandeWeele and Robins (2010) in the Discussion of the main text. Currently the reference only appears as a note in the Additional file 1.

   Authors' response: *The work by VandeWeele and Robins (2010) has the reference number 20 in the main text. We added a comment about this work in discussion. They proved that if the structural equations satisfy strong monotone conditions then it is possible to give a sign for a link: positively monotonically associated variables have positive correlation and negatively – negative correlation. Essentially the authors deal with expected correlations. They showed also that if the strong monotone condition will be substituted by weaker monotone condition then the rule of signs does not hold in general. In our case the monotone conditions*

appears from the comparisons of mean values, and there are the possibility to have unexpected links. Our aim is, starting from the definition of expected links, to prove the noise source of the appearance of unexpected links. Thus we prefer to mention the work instead of deep discussion of their results.

5) First paragraph in "Additional file 1, II.3. PUC represents 50 % of erroneous" - I think that the sentence "… are random such that … " should be changed to "… are mutually independent such that …".

Authors' response: *We decided to maintain the joint distribution of perturbed variables as it is. This noise distribution is a result of the noise propagation in a system, and it cannot be considered as a product distribution, because of nonzero covariances between them. The condition we posed on this distribution is: the half of their correlations of noise variables should be positive asymptotically.*

6) The authors should improve the quality of Additional file 1: Figure S1.

Authors' response: *Done*

7) The authors might want to provide a simple computational program to run the proposed method on real or simulated data sets. That might help not only to popularize the method, but also to clarify the idea for some readers.

Authors' response: *We add the algorithm in Additional file* 1 *and accompanying text with comments.*

Additional file

Additional file 1: Supporting results. (DOCX 1036 kb)

Acknowledgements
We thank Eric Zubriski for developing a code and running some simulations, Chris Sullivan, from Oregon State University for help in setting-up computation infrastructure at CGRB (Center for Genomic Research and Biocomputing). We also thank Amiran Dzutsev, Steve Ramsey, Lina Thomas and Jesse Zaneveld for critical reading of the manuscript.

Funding
AY was supported by FAPESP (grant 2012/06564-0); YK and AM are supported by NSF grant (award number: 1412557).

Availability of data materials
All the data used in the analysis is publicly available and accession numbers are provided in the text.

Authors' contributions
AY conceived original idea , developed mathematical model, supervised simulation analyses, and drafted the manuscript; MAP analyzed economical and cancer data, performed simulations analysis with GeneNetWeaver and drafted the manuscript; YK proved generalized mathematical model, drafted corresponding part of manuscript; NS provided data for microbiota colonization, participated in analyses of cancer data and drafted manuscript; KLM participated in analysis of cancer data; XD analyzed cancer and gene silencing data; AM conceived original idea, analyzed data, supervised and led the whole project, drafted the manuscript. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

Consent for publication
Not applicable.

Ethical Approval and Consent to participate
Not applicable.

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Received: 6 February 2016 Accepted: 1 October 2016
Published online: 13 October 2016

References
1. Butte AJ, Tamayo P, Slonim D, Golub TR, Kohane IS. Discovering functional relationships between RNA expression and chemotherapeutic susceptibility using relevance networks. Proc Natl Acad Sci U S A. 2000;97(22):12182–6.
2. Opgen-Rhein R, Strimmer K. From correlation to causation networks: a simple approximate learning algorithm and its application to high-dimensional plant gene expression data. BMC Syst Biol. 2007;1:37.
3. Pe'er D, Hacohen N. Principles and strategies for developing network models in cancer. Cell. 2011;144(6):864–73.
4. Shulzhenko N, Morgun A, Hsiao W, Battle M, Yao M, Gavrilova O, Orandle M, Mayer L, Macpherson AJ, McCoy KD, et al. Crosstalk between B lymphocytes, microbiota and the intestinal epithelium governs immunity versus metabolism in the gut. Nat Med. 2011;17(12):1585–93.
5. Margolin AA, Nemenman I, Basso K, Wiggins C, Stolovitzky G, Favera RD, Califano A. ARACNE: an algorithm for the reconstruction of gene regulatory networks in a mammalian cellular context. BMC Bioinf. 2006;7 Suppl 1:S7.
6. Stuart JM, Segal E, Koller D, Kim SK. A gene-coexpression network for global discovery of conserved genetic modules. Science. 2003;302(5643):249–55.
7. Barabasi AL, Oltvai ZN. Network biology: understanding the cell's functional organization. Nat Rev Genet. 2004;5(2):101–13.
8. Schadt EE. Molecular networks as sensors and drivers of common human diseases. Nature. 2009;461(7261):218–23.
9. Akavia UD, Litvin O, Kim J, Sanchez-Garcia F, Kotliar D, Causton HC, Pochanard P, Mozes E, Garraway LA, Pe'er D. An integrated approach to uncover drivers of cancer. Cell. 2010;143(6):1005–17.
10. Greer R, Dong X, Morgun A, Shulzhenko N. Investigating a holobiont: Microbiota perturbations and transkingdom networks. Gut Microbes. 2016;7(2):126–35.
11. Keller MP, Choi Y, Wang P, Davis DB, Rabaglia ME, Oler AT, Stapleton DS, Argmann C, Schueler KL, Edwards S, et al. A gene expression network model of type 2 diabetes links cell cycle regulation in islets with diabetes susceptibility. Genome Res. 2008;18(5):706–16.
12. Greer RL, Dong X, Zielke R, Vasquez-Perez S, Peremyslova E, Sikora AE, Morgun A, Shulzhenko N. Akkermansia muciniphila mediates negative effects of IFNg on glucose metabolism. Nat Commun. 2016;7:13329. doi:10.1038/ncomms13329.
13. Morgun A, Dzutsev A, Dong X, Greer RL, Sexton DJ, Ravel J, Schuster M, Hsiao W, Matzinger P, Shulzhenko N. Uncovering effects of antibiotics on the host and microbiota using transkingdom gene networks. Gut. 2015; 64(11):1732–43. doi:10.1136/gutjnl-2014-308820.
14. Carro MS, Lim WK, Alvarez MJ, Bollo RJ, Zhao X, Snyder EY, Sulman EP, Anne SL, Doetsch F, Colman H, et al. The transcriptional network for mesenchymal transformation of brain tumours. Nature. 2010;463(7279):318–25.
15. Sanchez-Garcia F, Villagrasa P, Matsui J, Kotliar D, Castro V, Akavia UD, Chen BJ, Saucedo-Cuevas L, Rodriguez Barrueco R, Llobet-Navas D, et al. Integration of genomic data enables selective discovery of breast cancer drivers. Cell. 2014;159(6):1461–75.
16. Mine KL, Shulzhenko N, Yambartsev A, Rochman M, Sanson GF, Lando M, Varma S, Skinner J, Volfovsky N, Deng T, et al. Gene network reconstruction

reveals cell cycle and antiviral genes as major drivers of cervical cancer. Nat Commun. 2013;4:1806.
17. Pearl J. An introduction to causal inference. Int J Biostat. 2010;6(2):Article 7.
18. Pearl J. Causality: models, reasoning and inference, vol. 29. Cambridge: Cambridge Univ Press; 2000.
19. Reichenbach H. The direction of time, vol. 65. Oakland: Univ of California Press; 1991.
20. Thomas LD, Fossaluza V, Yambartsev A. Building complex networks through classical and Bayesian statistics-A comparison. In: XI brazilian meeting on bayesian statistics: EBEB 2012, 2012, Amparo-SP. AIP Conference Proceedings. arXiv:14092833. 2012;1490:323–31.
21. Schaffter T, Marbach D, Floreano D. GeneNetWeaver: in silico benchmark generation and performance profiling of network inference methods. Bioinformatics. 2011;27(16):2263–70.
22. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc Ser B. 1995;57(1): 289–300.
23. Genovese C, Wasserman L. Operating characteristics and extensions of the false discovery rate procedure. J R Stat Soc Series B Stat Methodology. 2002;64(3):499–517.
24. Efron B, Tibshirani R, Storey JD, Tusher V. Empirical Bayes analysis of a microarray experiment. J Am Stat Assoc. 2001;96(456):1151–60.
25. Storey JD. A direct approach to false discovery rates. J R Stat Soc Series B Stat Methodology. 2002;64(3):479–98.
26. Storey JD. The positive false discovery rate: A Bayesian interpretation and the q-value. Ann Stat. 2003;31(6):2013–35.
27. Benjamini Y, Yekutieli D. The control of the false discovery rate in multiple testing under dependency. Annals of statistics 2001:1165–1188.
28. Skinner J, Kotliarov Y, Varma S, Mine KL, Yambartsev A, Simon R, Huyen Y, Morgun A. Construct and Compare Gene Coexpression Networks with DAPfinder and DAPview. BMC Bioinf. 2011;12:286.
29. VanderWeele TJ, Robins JM. Signed directed acyclic graphs for causal inference. J R Stat Soc Series B Stat Methodology. 2010;72(1):111–27.

Additional file 1

Unexpected links reflect the noise in networks

Authors: Anatoly Yambartsev[1], Michael A. Perlin[2], Yevgeniy Kovchegov[3], Natalia Shulzhenko[4], Karina L. Mine[5], Xiaoxi Dong[2], Andrey Morgun[2].

I. Experimental procedures

I.1. Statistically significant correlations between differentially expressed genes (DEGs) and show expected signs

In our recent study (Nature Commun. 2013;4:1806) we have shown that key drivers of cervical carcinogenesis are located in regions of frequent chromosomal aberrations and that these genes cause most of the alteration in gene expression in cervical cancer. Therefore, in order to evaluate whether statistically significant correlations between DEGs which result from known causal relations follow our prediction we performed the following analysis:

First, we selected two groups of genes from DEGs discovered in our previous study: 1) genes in which it has been determined that chromosomal aberrations are responsible for the change in regulation; and 2) genes located in regions in which aberrations are rare, defined by FqG – FqL between -0.1 and 0.1 (Figure S1). Next, we analyzed gene co-expression in tumors samples in order to find correlations between those two groups of DEGs. We found 626 correlated gene-gene pairs with FDR 5%. We used data from the following datasets for our meta-analysis (performed as described in Nature Commun. 2013;4:1806): GSE26342, GSE7410, GSE9750, GSE6791, GSE7803. In brief, we calculated correlations within the tumor samples of each dataset. If correlations presented the same sign in all datasets, then we calculated a corresponding Fisher meta-analysis p-value. We then computed the FDR for these correlations. The results provided support to our hypothesis that significant correlations should have “expected” signs. Indeed, 95% (594 of 626 total pairs) of significant correlations had expected signs.

I.2. PUC correlates with FDR in macroeconomic data

The macroeconomic data we analyzed was a combination of all data for the year 2008 on official UN member states in the following World Bank databases: Doing Business, Education Statistics, Gender Statistics, Health and Nutrition Population Statistics, IDA Results Measurement System; Poverty and Inequality Database, World Development Indicators and Global Development Finance. From this data set, we removed all duplications of macroeconomic parameters, as well as all parameters for which data only

existed for ≤ 25 countries. Of the remaining parameters, we used a dip test to determine those which were non-unimodal with a p-value of $< 2.2\times10^{-16}$. From the resulting set of parameters, we selected several with bimodal distributions, each of which we used to define two distinct states of a macroeconomic parameter network. We then computed PUC for parameter correlation networks at different FDR thresholds using each of these definitions. The results of these calculations are shown in Figure S2.

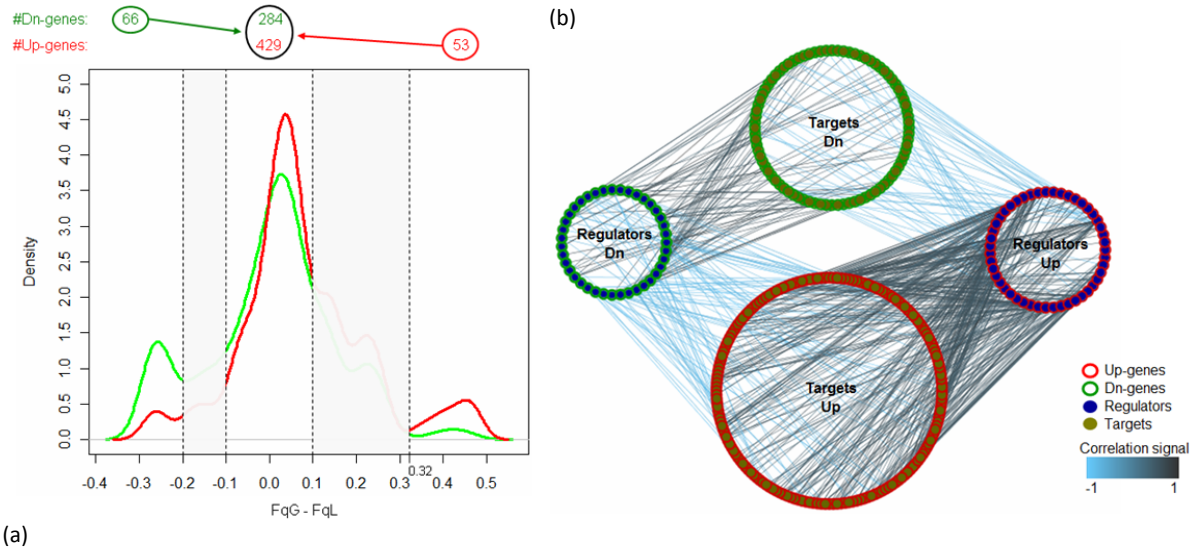


Figure S1: Genes directly regulated by chromosomal aberrations can also in turn regulate genes located outside of the aberrations. (a) Genes regulated by chromosomal aberrations in the expected direction (located in the regions $FqG - FqL < -0.2$ or $FqG - FqL > 0.3$) were considered as potential regulators, and genes located within the regions of very rare aberrations ($|FqG - FqL| \leq 0.1$) were considered to be potential targets. The green (red) line represents up-regulated (down-regulated) genes. (b) The reconstructed regulatory network with correlations in agreement with gene expression. The two green (red/purple) circles are made of up down-regulated (up-regulated) nodes, the middle (side) circles are made up of targets (regulators), and the black (cyan) lines represent positive (negative) correlations.

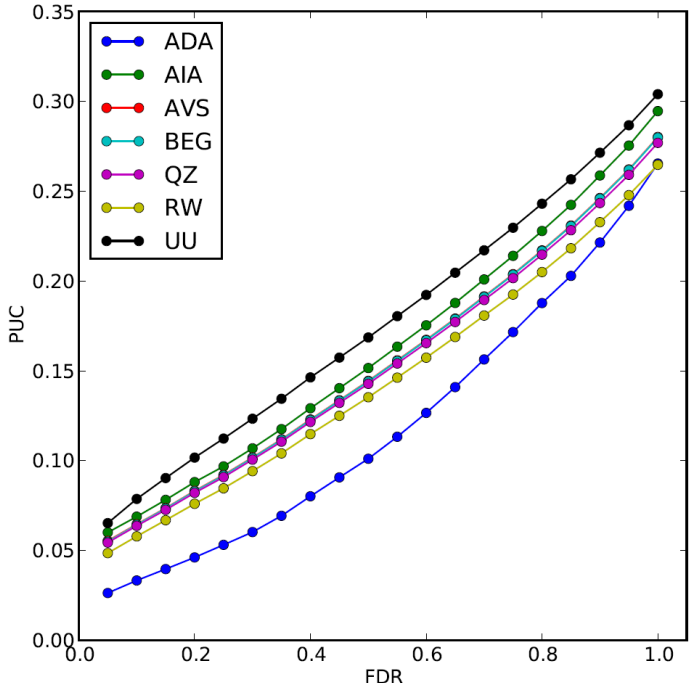


Figure S2: PUC and FDR correlate strongly when reconstructing macroeconomic networks using various bimodal parameters to define system states. Parameters shown: ADA - Duration of compulsory education; AIA - Cause of death, by communicable diseases and maternal, prenatal and nutrition conditions (% of total); AVS - Manufactures exports (% of merchandise exports); BEG - Educational expenditure in pre-primary as % of total educational expenditure; QZ - Private credit bureau coverage (% of adults); RW - Strength of legal rights index; UU Passenger cars (per 1,000 people)

II. Theoretical basis.

Here we provide some formal definitions of concepts used in the paper and all necessary proofs. This section consists of four parts: 1) we introduce the mathematical machinery for PUC using Bayesian networks; 2) we generalize the previous formalism to handle a broader set of cases; 3) we demonstrate that PUC reflects half of total network error; and 4) we address concerns with network non-monotonicity.

II.1. PUC on Bayesian networks.

In order to apply the new concept of noise estimator we use Bayesian Networks as a convenient model for gene expression. Let $G = (V, E)$ be some network, which is directed acyclic graph (DAG). Any edge $e \in E$ is an ordered pair of vertices $e = (v, w)$: and direction of edge is from the first vertex v to the second vertex w. We assume that the graph is weighted graph – any edge $e = (v, w)$ has its labels (weight), c_{vw} , which is some real number $c_{vw} \in \mathbb{R}$. For any node v we associate the set of parents of the node v:

$$pa(v) \coloneqq \{w \in V : (w, v) \in E\} \quad (1)$$

We define the set of root-nodes for the graph G:

$$gf(G) \coloneqq \{v \in V : pa(v) = \emptyset\} \quad (2)$$

With any node (gene) $v \in V$ we associate the random variable (gene expression) M_v. The random variables satisfy the following linear relations (structure equations): for any $v \notin gf(G)$

$$M_v = \sum_{w \in pa(v)} c_{wv} M_w + \varepsilon_v, \quad (3)$$

where ε_v are i.i.d. random variable (intrinsic noise) with mean 0 and variance σ^2. Moreover, for simplicity we suppose that there exists only one grandfather $|gf(G)| = 1$ and let us denote it as a vertex o.

A path $\pi(v,w)$ of length n from a vertex v to a vertex w is a sequence of edges $e_i = (v_i, v_{i+1}), i = 1, \dots, n-1$, with $v_1 = v$ and $v_n = w$. The weight of the path $W(\pi(v,w))$ is the product of weights of edges from this path:

$$W\big(\pi(v,w)\big) \coloneqq \prod_i c_{v_i, v_{i+1}} \quad (4)$$

Let $\Pi(v,w)$ be the set of all paths connecting nodes v and w. And let

$$W(v,w) \coloneqq \sum_{\pi \in \Pi(v,w)} W\big(\pi(v,w)\big) \quad (5)$$

The graph coupled with expressions we consider as a model of regulatory signaling paths system. The distribution of expressions within the system is determined by the topology of the graph, weights and the distribution of expressions of root-nodes.

For example, let o be the root-node vertex and $M_o^{(P)}$ and $M_o^{(Q)}$ its expressions in these two different states. Denote by d^2, d the variance and standard deviation for root-node expression in two states, and suppose that they do not depend on the state: $d^2 \coloneqq \mathbb{V}\mathrm{ar}\left(M_o^{(P)}\right) = \mathbb{V}\mathrm{ar}\left(M_o^{(Q)}\right)$. Denote the mean changes in expression of root-node´s gene as $\Delta_o = \mathbb{E}M_o^{(P)} - \mathbb{E}M_o^{(Q)}$. Expression for any non-root-node vertex v can be expressed for any state $S \in \{P, Q\}$ by the formula:

$$M_v^{(S)} = M_o^{(S)} W(o,v) + \sum_{w \in V \setminus o} \varepsilon_w^{(S)} W(w,v) \quad (6)$$

The mean change in the expression of a gene $v \in V \setminus o$ is given by:

$$\Delta_{\boldsymbol{v}} \coloneqq \mathbb{E}M_v^{(P)} - \mathbb{E}M_v^{(Q)} = \Delta_o W(o,v). \quad (7)$$

Moreover, for any $S \in \{P, Q\}$:

$$cov\left(M_v^{(S)}, M_w^{(S)}\right) = d^2 W(o,v) W(o,w) + \sum_{v' \in V \setminus o} \sigma_{v'}^2 W(v',v) W(v',w) \quad (8)$$

*Definition. We say that a pair of genes $v, w \in V$ satisfy **expected correlation inequality** if and only if*

$$\Delta_v \Delta_w \, cov\left(M_v^{(P)}, M_w^{(P)}\right) \geq 0, \ \Delta_v \Delta_w \, cov\left(M_v^{(Q)}, M_w^{(Q)}\right) \geq 0 \qquad (9)$$

*If (9) holds then we say that the two gene expressions $M_v^{(P)}, M_w^{(P)}$ or $M_v^{(Q)}, M_w^{(Q)}$ have **expected correlations**. If one or both expected correlations inequalities are not satisfied, we say that $M_v^{(P)}, M_w^{(P)}$ or $M_v^{(Q)}, M_w^{(Q)}$ have **unexpected correlations**.*

Note that in the considered model, by (8) the co-variations in (9) do not depend on a state: $cov\left(M_v^{(P)}, M_w^{(P)}\right) = cov\left(M_v^{(Q)}, M_w^{(Q)}\right)$. This independence means that we can use co-variation only in one state in our definition. In this case the following statement takes place.

Lemma 1. For any finite DAG network with linear relations between variables there exists some σ_0^2 such that if $\sigma_v^2 < \sigma_0^2$ for any $v \in V$, then there are no unexpected correlations into the network.

Proof. Direct from formulas (7), (8). By definition (9) and by representations (7), (8) we have:

$$\Delta_v \Delta_w \, cov\left(M_v^{(P)}, M_w^{(P)}\right) =$$
$$\Delta_o^2 W(o,v) W(o,w)\left(d^2 W(o,v) W(o,w) + \sum_{v' \neq o} \sigma_{v'}^2 W(v',v) W(v',w)\right) =$$
$$\Delta_o^2 d^2 W^2(o,v) W^2(o,w) + \Delta_o^2 W(o,v) W(o,w) \sum_{v' \neq o} \sigma_{v'}^2 W(v',v) W(v',w) \qquad (10)$$

Here the first term is necessarily positive and the second can be made arbitrarily small by choice of $\sigma_{v'}^2$ small enough for all $v' \in V$. Thus $\Delta_v \Delta_w \, cov\left(M_v^{(P)}, M_w^{(P)}\right)$ can always be made positive (implying that there are no unexpected correlations) by a choice of a sufficiently small variance σ_o^2. This statement is precisely Lemma 1.

The formula (8) shows that any link/correlation between two nodes in a network can be represented as a sum of two parts: *causal propagation* from causal node and *noise propagation* part:

$$cov\left(M_v^{(S)}, M_w^{(S)}\right) = \underbrace{d^2 W(o,v) W(o,w)}_{causal\ propagation} + \underbrace{\sum_{v' \neq o} \sigma_{v'}^2 W(v',v) W(v',w)}_{noise\ propagation} \qquad (11)$$

Here, it is easy to see that if the root-node variance d^2 increases, then the causal propagation will determine the sign of the covariance after some threshold. It means that it determines a link to be expected or unexpected.

Moreover, Lemma 1 says that if we observe in such regulation networks (DAGs with linear relationships between variables) unexpected correlations, it means that they appeared as a result of noise propagation within the network. Thus, the proportion of unexpected correlation reflects the noise level in a network (to the extent to which this mathematical framework, or that generalized in Section II.2 below, accurately reflects the system being modeled).

Note 1. The concept of expected correlations was also observed in VanderWeele and Robins, 2010, as a rule governing the relationship between monotonic links and the sign of covariance between variables.

Note 2. The linear relations between variables can be generalized: the expression $X_v = f_v\left(\{X_{v'}\}_{v' \in pa(v)};\ \varepsilon_v\right)$*, where* f_v *is a monotone function, and* ε_v *is internal network noise. If structural functions are monotonic function, then the lemma holds also.*

Estimation of noise. Error estimation is based on the following: if two genes belong to two independent subnetworks (see Figure 2a), then the correlation between their respective expression levels has to be equal to 0. Observable correlations, however, can be significantly different from 0 due to noise, in which case the observable correlation is positive (or negative) in roughly 50% of the cases (see formula (22)). On average, then, half of all random correlations between any pair of genes from unrelated subnetworks can be classified as unexpected, as in (9). Thus $2 \cdot PUC$ can be used as an estimate of total error.

Moreover, it is possible to prove for tree like graphs that within one network the noise propagation (see the formula (12)) has the same property as stated in formula (22). Indeed, the representation (6) means that any variable $M_v^{(S)}$ can be decomposed into the causal component $X_o^{(S)} W(o,v)$ and the noise component $\xi_v^{(S)} := \sum_{w \in V \setminus o} \varepsilon_w^{(S)} W(w,v)$. Then the covariance between $\xi_v^{(S)}$ and $\xi_w^{(S)}$ can be calculated exactly (compare with formula (10))

$$cov\left(\xi_v^{(S)}, \xi_w^{(S)}\right) = \sum_{u \in V} \sigma_u^2 W(u,v) W(u,w). \qquad (12)$$

If c_{vw} are mutually independent, identically distributed, with positive probabilities for being positive or negative, then the covariance (12) for any $S \in \{P, Q\}$ will be negative approximately in half of all cases.

Note that our results are related to mathematical aspect of the phenomenon. In practice we deals with observations, and we should estimate the corresponding variables such as covariances and mean values. Let

$$X^{(P)} = \left(x_{ij}^{(P)}, i = 1, \dots, p, j = 1, \dots, n^{(P)}\right)$$

$$X^{(Q)} = \left(x_{ij'}^{(Q)}, i = 1, \dots, p, j' = 1, \dots, n^{(Q)}\right)$$

be observation matrices for two equilibrium states P and Q, where $x_{ij}^{(P)}$ is the observed expression level of i-th gene, $i = 1, \dots, p$, in state P in j-th sample, $j = 1, \dots, n^{(P)}$ and $n^{(P)}$ is the number of samples we have for the state P; and, correspondingly, $x_{ij}^{(Q)}$ is the observed expression level of i-th gene, $i = 1, \dots, p$, in state Q in j'-th sample, $j' = 1, \dots, n^{(Q)}$ and $n^{(Q)}$ is the number of samples we have for the state Q. Denote $\bar{x}_i^{(P)}$ and $\bar{x}_i^{(Q)}$ arithmetical mean of i-th gene expression if state P and Q correspondingly. Estimation of mean difference of i-th gene, Δ_i, between states we denote $\hat{\Delta}_i := \bar{x}_i^{(P)} - \bar{x}_i^{(Q)}$. And denote $r_{ii'}^{(P)}, r_{ii'}^{(Q)}$ empirical correlation between gene i and i' in state P and Q. The statistical analogy of our definition of expected and unexpected links will be the following.

*Definition. An edge $e \in E$ is called an **expected link** between genes $i, j \in \{1, \dots, p\}$ if and only if $\hat{\Delta}_i \hat{\Delta}_j r_{ij}^{(P)} > 0$ and $\hat{\Delta}_i \hat{\Delta}_j r_{ij}^{(Q)} > 0$. An edge which is not an expected link is said to be an **unexpected link**.*

Moreover, in practice we calculate correlation matrix only for one of the state of main interest. For example, in cancer study, first we choose only differentially expressed genes, i.e. the genes which have statistical significant difference $\hat{\Delta}_i$, and calculate correlations using only observation sampled from cancer group. Indeed, in practice if for some genes i and j in a state P the condition $\hat{\Delta}_i \hat{\Delta}_j r_{ij}^{(P)} > 0$ holds true, then extremely rarely cases when the condition $\hat{\Delta}_i \hat{\Delta}_j r_{ij}^{(Q)} > 0$ does not hold in state Q. Let the state P be the our main interest state, then the PUC calculation will be the following.

input: $X^{(P)}, X^{(Q)}$;

1. choose a set V of differentially expressed genes i: $\hat{\Delta}_i \neq 0$;

2. calculate correlation matrix with elements $r_{ij}^{(P)}$ for i, j from the set V;

3. choose a set of edges E of statistically significant correlations (i, j): $r_{ij}^{(P)} \neq 0$;

   let $|E|$ be the number of edges into the set of edges E;

4. choose a set U of unexpected links from the set E: $(i,j) \in \mathrm{E}$ s.t. $\hat{\Delta}_i \hat{\Delta}_j r_{ij}^{(P)} < 0$;

let $|U|$ be the number of edges into the set of edges U;

output: $PUC = |U|/|E|$; error= $|U|/(|E| - |U|)$.

II.2. Definitions and generalization.

Here we study the concept of unexpected links in a more general framework. The positive and negative correlation inequalities are an active research direction in the field of probability and statistical mechanics. We believe these inequalities will allow us to generalize the concept of unexpected correlations in the PUC method. The following framework connects FKG (Fortuin–Kasteleyn–Ginibre) inequality in Statistical Mechanics to the concept of expected and unexpected links.

Let Ω be the underlying sample space of a biological system. As an example of a biological system we consider a gene regulatory network, where Ω represents the set of all possible gene expression configurations. We can suppose that the state space Ω has an ordering (or partial ordering) "$\prec$" assigned to pairs of its elements.

Definition. *A random variable $X = X(\omega)$ is said to be increasing if $\omega \prec \omega'$ implies $X(\omega) < X(\omega')$. Similarly, a random variable is decreasing if $\omega \prec \omega'$ implies $X(\omega) > X(\omega')$. Both types of random variables, increasing and decreasing, are said to be monotone random variables.*

In the field of statistical mechanics and probabilistic combinatory, the FKG inequality (Fortuin–Kasteleyn–Ginibre inequality) explains most of the results involving monotone random variables and monotone (increasing or decreasing) events. It states that for two increasing random variables X and Y,

$$\mathbb{E}(XY) \geq \mathbb{E}(X)\mathbb{E}(Y) \qquad (13)$$

In some applications, such as percolation models, partial ordering of Ω is sufficient for the FKG to hold (Grimmett, 1999). Many important results in applied mathematics and physics, such as the exact value of critical probability in two-dimensional percolation models, would have been impossible without the FKG inequality.

Let $G = (V, E)$ be a graph (network) with vertices (nodes) V and edges E. Nodes $v \in V$ represent the genes. Let $X_v(\omega)$ be monotone functions (random variables) assigned to each node $v \in V$. Here X_v represents the noiseless gene expressions. In this framework it

is convenient represent the state system as a probability measure. Consider two probability measures P and Q over Ω such that for all $\omega \in \Omega$:

$$P(\sigma \in \Omega: \sigma \prec \omega) \geq Q(\sigma \in \Omega: \sigma \prec \omega) \quad (14)$$

Here P and Q correspond to the two states of a biological system. Let us denote, as before, $\Delta_v := \mathbb{E}_P[X_v] - \mathbb{E}_Q[X_v]$. We repeat the definition of expected and unexpected links.

*Definition. We say that random variables X_v and X_u modeling gene expressions in a pair of genes satisfy **expected correlation inequality** if and only if*

$$\Delta_v \, \Delta_u \, cov_P(X_v, X_u) \geq 0, \; \Delta_v \, \Delta_u \, cov_Q(X_v, X_u) \geq 0, \quad (15)$$

*in which case we say that the two gene expressions X_v and X_u have **expected correlations**. If one or both expected correlations inequalities are not satisfied, we say that X_v and X_u have **unexpected correlations**.*

Lemma 2. If X_v and X_u are monotone functions, and probability measures P and Q satisfy the condition (13), then X_v and X_u satisfy expected correlation inequality (or X_v and X_u have expected correlations).

Proof. Indeed, if X_v is an increasing (decreasing) variable, then $\Delta_v \leq 0$ ($\Delta_v \geq 0$). Now, if both X_u and X_v are either increasing or decreasing the FKG inequality (13) implies non-negative correlations, so that for any state $S \in \{P, Q\}$

$$cov_S(X_u, X_v) := \mathbb{E}_S[X_u X_v] - \mathbb{E}_S[X_u]\mathbb{E}_S[X_v] \geq 0, \; \forall \, u, v \in V, \quad (16)$$

which implies expected correlation inequalities (15).

Similarly, if one of the two variables (i.e. X_u or X_v) is increasing while the other is decreasing, the FKG inequality (13) implies non-positive correlations, such that for any state $S \in \{P, Q\}$,

$$cov_S(X_u, X_v) := \mathbb{E}_S[X_u X_v] - \mathbb{E}_S[X_u]\mathbb{E}_S[X_v] \leq 0, \; \forall \, u, v \in V \quad (17)$$

implying (15) hold once again. It proves the Lemma 2. □

Next, let ξ_v denote the errors for each node $v \in V$. We assume that the random variables $\xi_v, v \in V$ are functions over a probability space Ξ, independent from any probability

measure over Ω, such as P and Q. Let μ be the joint distribution of $\xi_v, v \in V$ and $\mathbb{E}_\mu[\xi_v] = 0$ for any $v \in V$. The measured gene expression we quantify as a random variable

$$M_v = X_v + \xi_v, \quad v \in V, \tag{18}$$

over the product space $\Omega \times \Xi$, and the two different states of a biological system correspond to two different probability product measures, $P \times \mu$ and $Q \times \mu$. Note that for any gene v:

$$\mathbb{E}_{P\times\mu}[M_v] - \mathbb{E}_{Q\times\mu}[M_v] = \mathbb{E}_P[X_v] - \mathbb{E}_Q[X_v] =: \Delta_v \tag{19}$$

The following Lemma is an analogous of the Lemma 1 for the general framework.

Lemma 3. If the variances of errors $\sigma_v^2 = Var(\xi_v)$ are small enough for all $v \in V$, then the pairs of measured gene expression M_v will also satisfy the inequalities (15). Thus in the noiseless networks we foresee no unexpected correlations.

Proof. The proof is a direct consequence of the covariance calculation.

$$cov_{S\times\mu}(M_u, M_v) = cov_{S\times\mu}(X_u + \xi_u, X_v + \xi_v) = cov_S(X_u, X_v) + cov_\mu(\xi_u, \xi_v) \tag{20}$$

By Cauchy-Schwarz inequality

$$|cov_S(\xi_u, \xi_v)| \le \sigma_u \sigma_v \tag{21}$$

the second covariance in (19) can be made so small that the sign of $cov_S(M_u, M_v)$ and the sign of $cov_S(X_u, X_v)$ will coincide. This proves Lemma. □

However in the noisy networks, the expected correlations rule (14) can be violated. Here the fraction of edges (u, v) violating (15) that we call the Proportion of the Unexpected Correlations (PUC) becomes an estimator of the frequency of false edges.

II.3. PUC represents 50% of erroneous.

For any $u, v \in V$; $S \in \{P, Q\}$; and $\mu \in \Xi$, let us assume that the error random variables $\xi_v, v \in V$ have the following asymptotic property, $cov_\mu(\xi_u, \xi_v)$ is positive for half of the $\binom{|V|}{2}$ edges (u, v), and negative for the rest of the pairs:

$$\lim_{|V|\to\infty} \frac{\#\{(u,v):\, cov_\mu(\xi_u,\xi_v)>0\}}{\binom{|V|}{2}} = \lim_{|V|\to\infty} \frac{\#\{(u,v):\, cov_\mu(\xi_u,\xi_v)<0\}}{\binom{|V|}{2}} = \frac{1}{2}. \tag{22}$$

If the covariance $cov_{S\times\mu}(M_u, M_v)$ is of a different sign than $cov_S(X_u, X_v)$ (i.e. if a particular correlation (u, v) is unexpected), it must hold that (see (20)):

$$\frac{cov_{S\times\mu}(M_u,M_v)\, cov_S(X_u,X_v)}{\left(cov_\mu(\xi_u,\xi_v)\right)^2} = \left(\frac{cov_S(X_u,X_v)}{cov_\mu(\xi_u,\xi_v)}\right)^2 + \frac{cov_S(X_u,X_v)}{cov_\mu(\xi_u,\xi_v)} < 0. \quad (23)$$

This condition is of the form $R^2 + R < 0$, where $R = \frac{cov_S(X_u,X_v)}{cov_\mu(\xi_u,\xi_v)}$, which trivially has the solution:

$$\frac{1}{R} = \frac{cov_\mu(\xi_u,\xi_v)}{cov_S(X_u,X_v)} < -1. \quad (24)$$

The resulting inequality is satisfied under two conditions, which are thus requisite for a correlation to be unexpected, namely:

$$|cov_\mu(\xi_u,\xi_v)| > |cov_S(X_u,X_v)| \quad (25)$$

$$cov_\mu(\xi_u,\xi_v)cov_S(X_u,X_v) < 0 \quad (26)$$

The first condition (25) is interpreted as a drowning out of the causal link between two nodes by error; that is, the magnitude of error in the correlation between two nodes' expressions is greater than the magnitude of real correlation between them. The second condition (26) is interpreted as a counteracting of error to causal connections: the contribution to the empirical correlation between two nodes due to error must counteract the contribution due to causal mechanisms.

Condition (26) implies that, given the condition (22) for error distribution, PUC will statistically detect 50% of total false correlations for which the causal contribution is negligibly small, as the signs of the error and causal contribution are equally likely to be the same as they are to be opposite.

II.4. Unexpected correlations under non-monotonicity.

Here we prove the proposition in the conclusion about non-monotonic links. The statement says that a non-monotonic link between two nodes with an unexpected correlation cannot cause a transition between two distinct states of a network. We provide an extreme example of non-monotonicity, in which the dependence between two nodes changes in sign in the two states of a network (e.g. stimulation in one state of a biological system and inhibition in the other).

Assume we are given $n+2$ gene expressions in two biological state P and Q : $X_P, Y_P, X_{1,P}, \dots, X_{n,P}$ and $X_Q, Y_Q, X_{1,Q}, \dots, X_{n,Q}$. We assume linear (or almost linear) dependence of Y on X within any one given biological state, stated as follows: $Y_P =$

$\alpha_P X_P + \xi_P$ and $Y_Q = \alpha_Q X_Q + \xi_Q$, where ξ_P is a function of $X_{1,P}, \dots, X_{n,P}$, and ξ_Q is a function of $X_{1,Q}, \dots, X_{n,Q}$, and $\alpha_P \alpha_Q \neq 0$. We suppose that X_P (X_Q) and ξ_P (ξ_Q) are independent. Recall that all gene expression values are positive and remember that $\Delta X \coloneqq \mathbb{E}_P[X] - \mathbb{E}_Q[X] = \mathbb{E}[X_P] - \mathbb{E}[X_Q]$.

Lemma 4. Suppose $\alpha_P \alpha_Q < 0$ (implying that the relation between X and Y is non-monotonic), then:

(a) X and Y have unexpected correlations.
(b) The sign of ΔY may not depend on the sign of ΔX, but instead mostly depends on the sign of $\Delta \xi$.

Proof. Observe that, due to independence of X_P (X_Q) and ξ_P (ξ_Q):

$$cov_P(X, Y) = cov(X_P, Y_P) = \alpha_P Var[X_P], \quad (27)$$

$$cov_Q(X, Y) = cov\big(X_Q, Y_Q\big) = \alpha_Q Var[X_Q]. \quad (28)$$

Therefore, $cov_P(X, Y) cov_Q(X, Y) < 0$ (so that the expected correlation inequalities do not hold simultaneously) if and only if $\alpha_P \alpha_Q < 0$. This proves the item (a) of the lemma.

Let us prove (b). Without loss of generality, $cov_P(X, Y) < 0$, implying $\alpha_P < 0$ and $\alpha_Q > 0$. Hence:

$$\Delta Y = \mathbb{E}\big(Y_P - Y_Q\big) = \mathbb{E}\big(\alpha_P X_P - \alpha_Q X_Q\big) + \mathbb{E}(\xi_P - \xi_Q) \quad (29)$$

Note that $\mathbb{E}\big(\alpha_P X_P - \alpha_Q X_Q\big) < 0$ regardless of the values of X_P and X_Q (both of which are strictly positive). Thus in the case $\Delta \xi > 0$ the change ΔY will still be negative. The sign of ΔY will be positive only if $\Delta \xi \gg 0$. □

III. Simulations using GeneNetWeaver.

We tested PUC using GeneNetWeaver (GNW), a software package designed for rigorous testing of gene network inference methods. We used GNW to generate various networks ranging in size from 40 to 740 nodes, each broken into two disjoint subnetworks in a similar manner as with the previous simulations. Distinct equilibrium network states were made by performing a 50% knockdown on the node in each subnetwork with the most connections. Networks were simulated 100 times both stochastically and analytically. In the case of analytic simulations, in order to get distinct equilibria in different simulations all genes were given normally distributed microperturbations, i.e. proportional up/down regulations with mean 0 and a standard deviation of 1.25%. After each simulation, we selected those genes which were differentially expressed with FDR < 0.01%, and calculated correlations between them in each class separately. We computed PUC and true error for the resulting regulatory networks consisting of at least 20 nodes at various FDR cutoffs. The results are summarized in figures S3 a,b

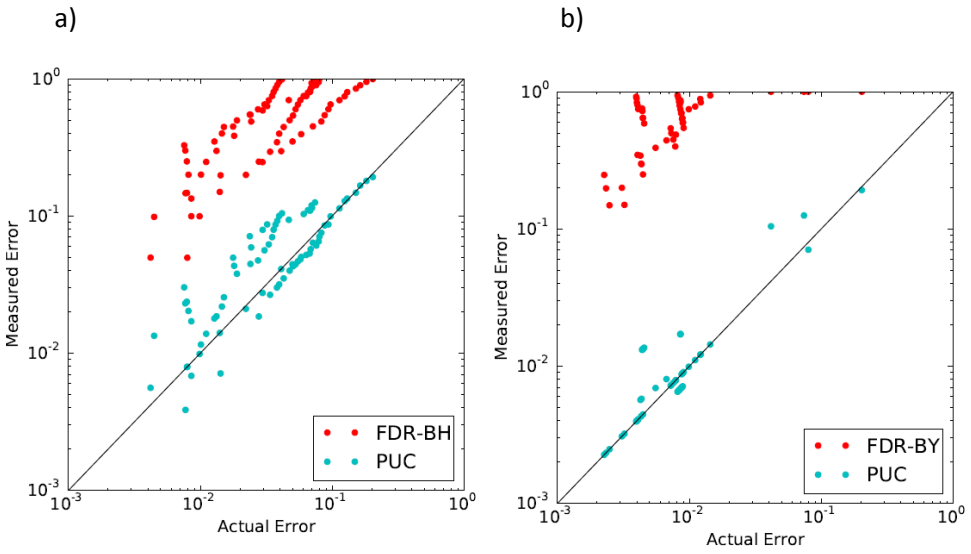
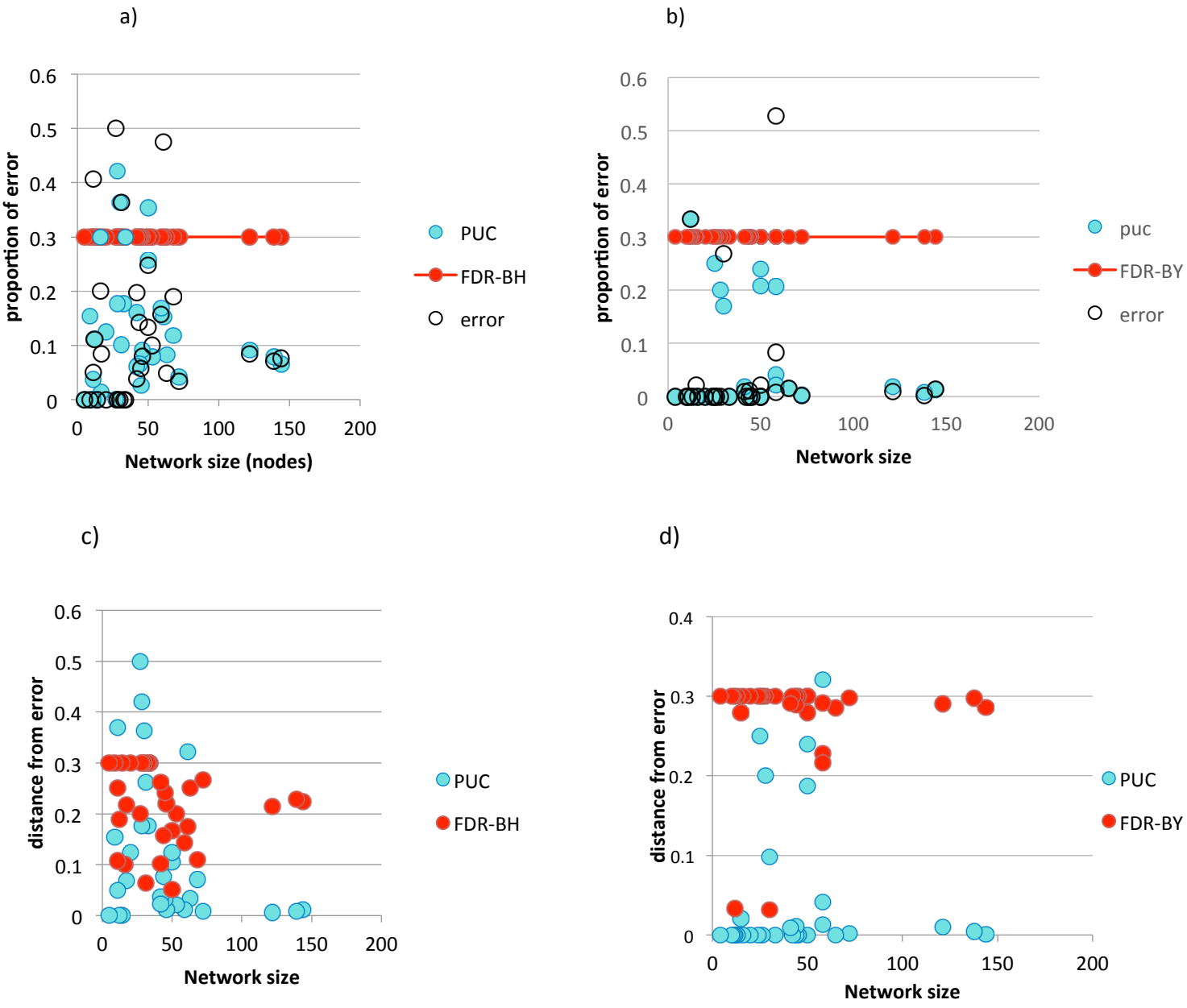


Figure S3. Comparison between PUC and FDR in networks simulated by GNW.

x axes represent actual error; y axes actual error (black line), PUC- blue dots, FDR –red dots (Benjamini-Hochberg- left panel; Benjamini-Yekutieli- right panel).

Figure S4. PUC, FDR-BH (a,c), FDR-BY (b,d) and error in networks of different sizes (number of nodes) simulated by GNW. Panels a) and b) show values for each metric (PUC, FDR or error). Panels c) and d) show the distance from error for FDR and PUC. Overall, PUC is closer to error than FDR.



List of datasets from BRB Array Tools Archive used for analysis:

GEO IDs:

GDS1021, GDS232, GDS408, GDS470, GDS484, GDS507, GDS531, GDS535, GDS536, GDS619, GDS690, GDS715, GDS760, GDS806, GDS838, GDS845-8, GDS884, GDS971, GDS978.

Note: for datasets that we could not find GEO ID we provide PUBMED IDs. All datasets were downloaded from BRB Array Tools Archive.

PUBMED IDs:

PMID:10359783, PMID:11707567, PMID:12925757, PMID:15548776, PMID:11707590.