GIMLET: Identifying Biological Modulators in Context-Specific Gene Regulation Using Local Energy Statistics

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Abstract. Regulation of transcription factor activity is dynamically changed across cellular conditions and disease subtypes. The identification of biological modulators contributing to context-specific gene regulation is one of the challenging tasks in systems biology, in order to understand and control cellular responses across different genetic backgrounds and environmental conditions. Previous approaches for the identification of biological modulators from gene expression data are restricted to the capturing of a particular type of a three-way dependency between a regulator, its target gene, and a modulator, and these methods cannot describe complex regulation structure, such as where multiple regulators, their target genes, and modulators are functionally related. Here, we propose a statistical method for the identification of biological modulators by capturing multivariate local dependencies, based on energy statistics, which is a class of statistics based on distances. Subsequently, out method assigns a measure of statistical significance to each candidate modulator by a permutation test. We compared our approach with a leading competitor for the identification of modulators, and illustrated its performance both through the simulation and real data analysis. GIMLET is implemented with R ($\geq$ 3.2.2) and is available from github (https://github.com/tshimam/GIMLET).

Keywords: Gene regulation · Modulator detection · Energy statistics · Distance correlation · Statistical test

1 Introduction

Regulation of gene expression is a process in which the expression of a particular gene can be either activated or repressed. Transcription factors (TFs) contribute

greatly to the process of gene regulation by binding to a specific DNA sequence in the promoter regions of their target genes and controlling their transcription. The responsiveness of a target gene expression to a TF is typically changed due to genetic variation or a change in the cellular environment. This modulation in gene-specific responsiveness is often caused by a specific factor called modulator at different levels, including the transcriptional, post-transcriptional and post-translational levels.

In the last decade, large international consortia, such as The Cancer Genome Atlas (TCGA) [1] and the International Cancer Genome Consortium (ICGC) [2], have generated large-scale gene expression profiles of different tumor types and catalogued their genetic alterations (recurrent mutations and copy number variations). Genome-wide association studies (GWAS) also have identified tens of thousands of human disease-associated variants and millions of single nucleotide polymorphisms [3]. However, it remains unknown if and how a lot of genetic alterations and variants interact with physical and functional interactions within cellular networks.

The identification of genetic alterations and variations that function as biological modulators and contribute to gene expression control is one of the challenging tasks in systems biology. Recently, sophisticated algorithms have been developed for this task which have successful applications in many areas [4,5,6,7,8,9]. For example, MINDy [4] formulates the problem of identifying modulators as a problem of testing if the expressions of a univariate transcription factor and its target gene, denoted by X and Y, are independent each other, conditioned on the expression levels of an univariate modulator denoted by Z in the framework of conditional mutual information. GEM [5] uses a linear regression model with the effects of interaction between X and Z to describe the relationships between X and Y modulated by Z. MIMOSA [6] considers a mixture model of X and Y from two different fractions based on Z. Note that these methods are designed to capture a particular type of three-way dependence where X, Y, and Z are univariate random variables. Therefore, they cannot capture multivariate dependencies where sets of random variables are associated with each other. Currently, no systematic mathematical framework is available for the identification of biological modulators of the complex gene regulation, such as combinatorial regulation, whether multiple transcription factors and modulators are functionally related.

In this study, we present a novel method, genome-wide identification of modulators using local energy statistical test (GIMLET), to overcome the challenges outlined above. GIMLET includes the following contributions.

1. GIMLET is mainly based on dependence coefficients from energy statistics for modeling the relationships between genes. This type of coefficients is a measure of statistical dependence between two random variables or two random vectors of arbitrary, not necessarily equal dimension. This enables to correlate the expression of sets of any size for TFs, their target genes, and modulators.

2. We provide a new dependence coefficient, called local distance correlation, to compare the difference of distance correlation at low and high values of given modulators, allowing the identification of all types of local dependence, such as non-monotone and non-linear relationships, between TFs and their target genes at the fixed point of modulators.
3. We develop a permutation-based approach to evaluate whether local distance correlation varies with modulators, which enables the discovery of modulators related with complex regulatory relationships, including synergistic and cooperative regulation, from a statistical point of view.

We describe our proposed framework and algorithm in Section 2. We also provide the efficiency of GIMLET using synthetic and real data in Sections 3 and 4.

2 GIMLET Methodology

2.1 Notations and Preliminaries

For a p-dimensional random vector $\boldsymbol{a}$, $|\boldsymbol{a}|$ represents its Euclidean norm. A collection of n i.i.d. observations for $\boldsymbol{a}$ is denoted as $\{\boldsymbol{a}_k; k = 1, \ldots, n\}$ where $\boldsymbol{a}_k = (a_1, \ldots, a_p)'$ represents the k-th sample.

The distance correlation [10] was introduced as a measurement of dependence between two random vectors $X \in \mathbf{R}^p$ and $Y \in \mathbf{R}^q$. It is based on the concept of distance covariance between X and Y, denoted by $\mathcal{V}^2(X, Y)$, which measures the distance between the joint characteristic function of (X, Y) and the product of the marginal characteristic functions as follows:

$$\mathcal{V}^2(X, Y) = \frac{1}{c_p c_q} \int_{\mathbf{R}^{p+q}} |f_{X,Y}(\boldsymbol{s}, \boldsymbol{t}) - f_X(\boldsymbol{s}) f_Y(\boldsymbol{t})|^2 w(\boldsymbol{s}, \boldsymbol{t}) d\boldsymbol{t} d\boldsymbol{s},$$

where $f_{X,Y}(\boldsymbol{s}, \boldsymbol{t})$, $f_X(\boldsymbol{s})$, and $f_Y(\boldsymbol{t})$ are the characteristic functions of (X,Y), X, and Y, respectively, and the weight function $w(\boldsymbol{s}, \boldsymbol{t}) = (c_p c_q |\boldsymbol{s}|^{1+p} |\boldsymbol{t}|^{1+q})^{-1}$ with constants $c_l = \pi^{(1+l)/2} / \Gamma((1 + l)/2)$ for $l \in \mathbf{N}$ is chosen to produce scale free and rotation invariant measure that does not go to zero for dependent variables.

The distance correlation $\mathcal{R}(X, Y)$ between X and Y is then defined as

$$\mathcal{R}(X, Y) = \frac{\mathcal{V}^2(X, Y)}{\sqrt{\mathcal{V}^2(X, X)\mathcal{V}^2(Y, Y)}}, \tag{1}$$

if $\mathcal{V}^2(X, X)\mathcal{V}^2(Y, Y) > 0$ and equals 0 otherwise. The remarkable properties of the distance correlation introduced by the equation (1) include $0 \leq \mathcal{R}(X, Y) \leq 1$ and $\mathcal{R}(X, Y) = 0$ if and only if X and Y are independent.

If we observe a collection $\{(\boldsymbol{x}_k, \boldsymbol{y}_k); k = 1, \ldots, n\}$ of n i.i.d. observations from the joint distribution of random vectors $X \in \mathbb{R}^p$ and $Y \in \mathbb{R}^q$, the empirical distance covariance between X and Y, denoted by $\mathcal{V}_n^2(X, Y)$, is then given by

$$\mathcal{V}_n^2(X, Y) = S_1(X, Y) + S_2(X, Y) - 2S_3(X, Y),$$

where

$$S_1(X,Y) = \frac{1}{n^2}\sum_{k,l=1}^{n} |\boldsymbol{x}_k - \boldsymbol{x}_l||\boldsymbol{y}_k - \boldsymbol{y}_l|,$$
$$S_2(X,Y) = \frac{1}{n^2}\sum_{k,l=1}^{n} |\boldsymbol{x}_k - \boldsymbol{x}_l| \frac{1}{n^2}\sum_{k,l=1}^{n} |\boldsymbol{y}_k - \boldsymbol{y}_l|,$$
$$S_3(X,Y) = \frac{1}{n^3}\sum_{k=1}^{n}\sum_{l,m=1}^{n} |\boldsymbol{x}_k - \boldsymbol{x}_l||\boldsymbol{y}_k - \boldsymbol{y}_m|.$$

The empirical distance correlation $\mathcal{R}_n(X,Y)$ is then

$$\mathcal{R}_n(X,Y) = \frac{\mathcal{V}_n^2(X,Y)}{\sqrt{\mathcal{V}_n^2(X,X)\mathcal{V}_n^2(Y,Y)}},$$

and satisfies $0 \leq \mathcal{R}_n(X,Y) \leq 1$.

2.2 Local distance correlation

We introduce a local estimator of the distance correlation evaluated at another random vector $Z = \boldsymbol{z}_\alpha \in \mathbb{R}^r$ as a local measurement of dependence between X and Y conditioning on $Z = \boldsymbol{z}_\alpha$ based on the observed data. We consider a collection $\{(\boldsymbol{x}_k, \boldsymbol{y}_k, \boldsymbol{z}_k) : k = 1, \ldots, n\}$ of n i.i.d. observations for random vectors X, Y, and Z. Let us denote $w_{k\alpha} = K_h(\boldsymbol{z}_k, \boldsymbol{z}_\alpha)$ satisfying $\sum_{k=1}^{n} w_{k\alpha} = 1$ as the new weight function based on the distance between two sample vectors $\boldsymbol{z}_k$ and $\boldsymbol{z}_\alpha$ where K_h is a specified kernel function with a bandwidth h.

Based on the definition of Nadaraya-Watson estimator [12,13] as a weighted averaging method, we define a local estimator of distance covariance conditioning on $Z = \boldsymbol{z}_\alpha$, using the weighted Euclidean distance as

$$\mathcal{V}_n^2(X,Y|Z=\boldsymbol{z}_\alpha) = S_1(X,Y|Z=\boldsymbol{z}_\alpha) + S_2(X,Y|Z=\boldsymbol{z}_\alpha) - 2S_3(X,Y|Z=\boldsymbol{z}_\alpha),$$

where

$$S_1(X,Y|Z=\boldsymbol{z}_\alpha) = \sum_{k,l=1}^{n} w_{k\alpha}w_{l\alpha}|\boldsymbol{x}_k - \boldsymbol{x}_l||\boldsymbol{y}_k - \boldsymbol{y}_l|,$$
$$S_2(X,Y|Z=\boldsymbol{z}_\alpha) = \sum_{k,l=1}^{n} w_{k\alpha}w_{l\alpha}|\boldsymbol{x}_k - \boldsymbol{x}_l| \sum_{k,l=1}^{n} w_{k\alpha}w_{l\alpha}|\boldsymbol{y}_k - \boldsymbol{y}_l|,$$
$$S_3(X,Y|Z=\boldsymbol{z}_\alpha) = \sum_{k=1}^{n} w_{k\alpha} \sum_{l,m=1}^{n} w_{l\alpha}w_{m\alpha}|\boldsymbol{x}_k - \boldsymbol{x}_l||\boldsymbol{y}_k - \boldsymbol{y}_m|.$$

Each sample of the neighborhood in the α-th sample is weighted according to its weighted Euclidean distance from $Z = \boldsymbol{z}_\alpha$. Points close to $Z = \boldsymbol{z}_\alpha$

have large weight, and points far from $Z = \boldsymbol{z}_\alpha$ have small weight. The kernel function K_h used in all of our examples is the Gaussian kernel function $K_h(\boldsymbol{z}_k, \boldsymbol{z}_\alpha) = \exp(-|\boldsymbol{z}_k - \boldsymbol{z}_\alpha|^2/h)$ where h is a bandwidth parameter that controls the smoothness of the fit. For a specific point $Z = \boldsymbol{z}_\alpha$, the nearest-neighbor bandwidth h is determined so that the local neighborhood contains the $q = \lfloor n\delta \rfloor$ closest samples to the α-th sample in the Euclidean distance of Z where $\delta \in (0, 1)$ is a tuning parameter that indicates the proportion of neighbors. Therefore, each local estimator is inferred with q observations that fall within the ball $B_\delta(\boldsymbol{z}_\alpha)$, centered at the α-th sample. We use a varying width parameter h, which reduces the problem of data sparsity by increasing the radius in the regions with fewer observations.

The empirical local estimator of the distance correlation, called local distance correlation, $\mathcal{R}_n(X, Y|Z = \boldsymbol{z}_\alpha)$ for given $Z = \boldsymbol{z}_\alpha$ is then defined by the equation

$$\mathcal{R}_n(X, Y|Z = \boldsymbol{z}_\alpha) = \frac{\mathcal{V}_n^2(X, Y|Z = \boldsymbol{z}_\alpha)}{\sqrt{\mathcal{V}_n^2(X, X|Z = \boldsymbol{z}_\alpha)\mathcal{V}_n^2(Y, Y|Z = \boldsymbol{z}_\alpha)}}, \tag{2}$$

if both $\mathcal{V}_n^2(X, X|Z = \boldsymbol{z}_\alpha)$ and $\mathcal{V}_n^2(Y, Y|Z = \boldsymbol{z}_\alpha)$ are strictly positive, and it is equal to zero otherwise.

2.3 Statistical hypothesis test for the identification of modulators

In the statistical hypothesis testing for the identification of modulators, it is of practical interest to assess whether the local dependence between X and Y varies with Z. This question can be formulated by:

$$H_0 : \mathcal{R}_n(X, Y|Z) = c \leftrightarrow H_1 : \mathcal{R}_n(X, Y|Z)) \neq c, \tag{3}$$

where $\mathcal{R}_n(X, Y|Z)$ is a function of Z and c is a constant.

For the calculation of the p-values of the local dependence between X and Y for each Z, we apply a permutation-based approach similar to the one used by [4]. Under the assumption that $\mathcal{R}_n(X, Y|Z)$ is a monotonic function of Z, we calculate the test statistic:

$$\Delta\mathcal{R}_n(X, Y|Z) = \left| \frac{1}{|\mathbb{U}_Z|} \sum_{k \in \mathbb{U}_Z} \mathcal{R}(X, Y|Z = \boldsymbol{z}_k) - \frac{1}{|\mathbb{L}_Z|} \sum_{k \in \mathbb{L}_Z} \mathcal{R}(X, Y|Z = \boldsymbol{z}_k) \right|, \tag{4}$$

where $\mathbb{U}_Z$ and $\mathbb{L}_Z$ are the index sets of the upper and lower points of Z, respectively. To assess the statistical significance of $\Delta\mathcal{R}_n(X, Y|Z)$, we generate a series of null hypotheses, and calculate the empirical p-value, using the following permutation procedures:

1. Permute the values of Z for all samples.
2. Re-calculate the test statistics using (4). Denote the null statistic of the l-th permutation by $\Delta\mathcal{R}_n^0(l)$.

3. Repeat steps 1-2 B times and calculate the empirical p-value for Z:

$$p_Z = \frac{1}{B}\sum_{l=1}^{B} I(\Delta\mathcal{R}_n(X,Y|Z) \leq \Delta\mathcal{R}_n^0(l)), \tag{5}$$

where the indicator function $I(A)$ equals 1 when the condition A is true and it equals 0 otherwise.

The statistical significance of Z, as expressed in (5), is the percent of null statistics, equally or more extreme than the observed statistic for given Z. Note that this empirical method directly couples both the minimal obtainable p-value and the resolution of the p-value to the number of permutations B.

The statistical significance of Z, as expressed in (5), is the percent of null statistics, equally or more extreme than the observed statistic for given Z. Note that this empirical method directly couples both the minimal obtainable p-value and the resolution of the p-value to the number of permutations B. Therefore, it requires a very large number of permutations to calculate the p-values when we want to accurately estimate small p-values. In order to compute more accurate p-values, we use a semi-parametric approach based on tail approximation [14,15]. The corrected empirical p-value $\tilde{p}_Z$, using the distribution tail approximation, is given by

$$\tilde{p}_Z = \begin{cases} p_Z & \text{if } \Delta\mathcal{R}_n(X,Y|Z) \leq \Delta\tilde{\mathcal{R}}_n^0 \\ \exp\left[-\lambda(\Delta\mathcal{R}_n(X,Y|Z) - \Delta\tilde{\mathcal{R}}_n^0)\right] & \text{otherwise} \end{cases}, \tag{6}$$

where λ is a scale parameter, and $\Delta\tilde{\mathcal{R}}_n^0$ is a threshold that we set to the 99-th percentile of null distributions. The parameter λ is estimated by the null statistics satisfying the condition $\Delta\mathcal{R}_n^0 > \Delta\tilde{\mathcal{R}}_n^0$.

3 Synthetic data results

We generated synthetic data and evaluated the performance of our method in order to gain insight into statistical power and type I error rate control in the identification of modulators, based on the hypothesis $H_0 : \mathcal{R}_n(X,Y|Z) = c \leftrightarrow H_1 : \mathcal{R}_n(X,Y|Z) \neq c$.

A simulation study was conducted as follows. An i.i.d. sample of (X,Y,Z) was generated using the endogenous switching regression model in the following three settings:

$$\begin{aligned} M_1 &: \quad Y = \mu(X,Z) + \sigma(Z)\varepsilon, \\ M_2 &: \quad Y = \mu(X,Z) + \sigma(Z_1 Z_2)\varepsilon, \\ M_3 &: \quad Y = \mu(X_1 X_2, Z) + \sigma(Z)\varepsilon, \end{aligned}$$

with

$$\mu(X_1X_2,Z) = \begin{cases} f_l(X_1X_2) & \text{if } Z > \theta_1 \\ 0 & \text{otherwise} \end{cases}, \quad \text{and} \quad \sigma(Z) = \begin{cases} \gamma_1 & \text{if } Z > \theta_2 \\ \gamma_2 & \text{otherwise} \end{cases},$$

where $X, X_1, X_2, Z, Z_1, Z_2 \sim U[0,1]$, $\varepsilon \sim N(0,1)$, μ and σ are the conditional mean and variance of Y depending on Z, and f_l is a function which determines a functional relationship between X and Y.

For a function $f_l(X)$, we considered the following eight different functional relationships:

$$\begin{array}{ll}
F_1\ (Line): & f_1(X) = X - 1/2, \\
F_2\ (Quadratic): & f_2(X) = 4(X-1/2)^2 - 1/2, \\
F_3\ (Cubic): & f_3(X) = 80(X-1/3)^3 - 12(X-1/3) - 7, \\
F_4\ (Sinusoid,\ 2\ periods): & f_4(X) = \sin(4\pi X), \\
F_5\ (Sinusoid,\ 8\ periods): & f_5(X) = \sin(16\pi X), \\
F_6\ (x^{1/4}): & f_6(X) = X^{1/4} - 1/2, \\
F_7\ (Circle): & f_7(X) = (2W-1)\sqrt{1-(2X-1)^2}, \\
F_8\ (Step): & f_8(X) = I(X > 1/2) - 1/2,
\end{array} \tag{7}$$

where $W \sim Bern(0.5)$. These functions were originally used in [16] to assess the statistical power against independence.

We set θ_1 and θ_2 to be 0.25 and 0.75, and γ_1 and γ_2 as follows:

$$\begin{aligned}
\gamma_1 &= \begin{cases} 1/6, \text{ if } f_l(X) = f_1(X) \text{ or } f_l(X) = f_3(X) \\ 1/2, \text{ otherwise} \end{cases}, \\
\gamma_2 &= \begin{cases} 1, \text{ if } f_l(X) = f_1(X) \text{ or } f_l(X) = f_3(X) \\ 3, \text{ otherwise} \end{cases}.
\end{aligned} \tag{8}$$

The scatter plots of the data obtained in these eight relationships are shown in Figure 1.

The first setting, M_1, was designed to find modulators in the traditional framework for the identification of modulators [4], where the expression value of a modulator $Z \in \mathbb{R}$ influences the dependence between the expression values of a transcription regulator $X \in \mathbb{R}$ and its target gene $Y \in \mathbb{R}$. The second and third settings, M_2 and M_3, were aimed at finding the modulators in the new conceptual framework investigated in this study: M_2 was intended for the combinatorial modulation where the expressions of two modulators $Z = (Z_1, Z_2)' \in \mathbb{R}^2$ influence the dependency between a transcription factor $X \in \mathbb{R}$ and its target gene $Y \in \mathbb{R}$, and M_3 was intended for combinatorial regulation, where the expression of a modulator $Z \in \mathbb{R}$ influences the dependency between two transcription factors $X = (X_1, X_2)' \in \mathbb{R}^2$ and their target gene $Y \in \mathbb{R}$, and both X_1 and X_2 are required for Y.

The identification of modulators was assessed with our method (GIMLET) and MINDy [4], one of the most widely used methods for this purpose. We note that MINDy cannot be directly applied for the identification of modulators in the settings M_2 and M_3, since MINDy is not designed for combinatorial modulation and regulation. In these simulations, all possible triplets were tested separately using MINDy, and the statistical significance was evaluated by Fisher's method, which is widely used to combine p-values. A hypothesis testing problem for the identification of modulators with varying sample sizes ($n = 100, 200, 500$) was

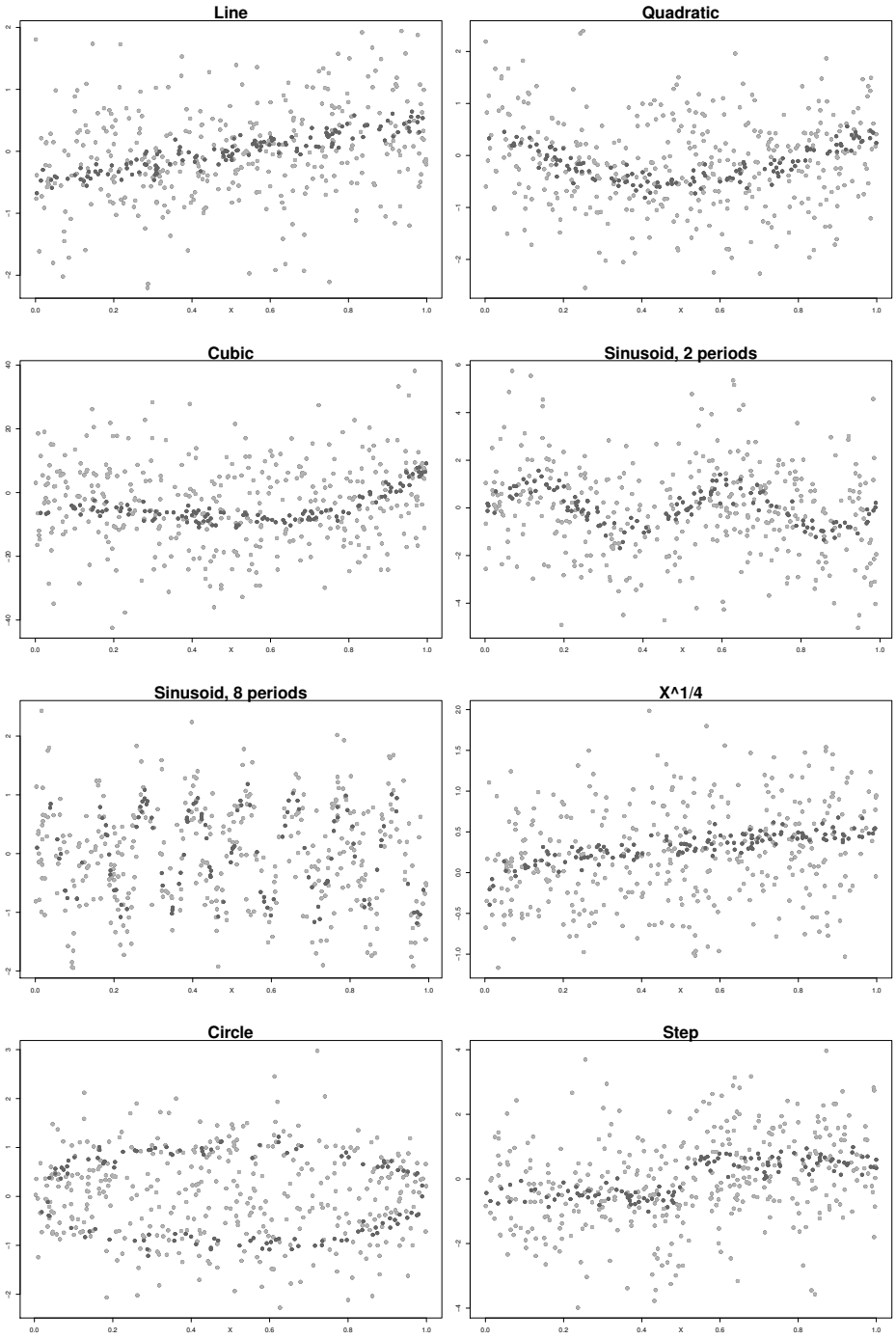

Fig. 1. Sample plots of the eight simulated relationships. Dark gray dots indicate samples with $Z > 0.75$, whereas light gray dots indicate samples with $Z \leq 0.75$.

simulated with 1,000 datasets generated for each of the above three settings. All tests were performed at the significance level $\alpha = 0.05$. Statistical power was estimated by the fraction of test statistics that were at least as large as the 95th percentile of the null distribution. The null distribution was calculated by $1,000$ permutations illustrate in Section 2. Type I error rate was estimated by calculating the power from data generated under the null hypothesis $H_0 : r(Z) = c$, which can be obtained by modifying the simulations where the random effect is set to be independent of Z. Theoretically, the type I error rate of the test should be equal to the significance level $\alpha = 0.05$.

Table 1. The statistical power of GIMLET and MINDy using synthetic data with different sample sizes ($n = 100, 200, 500$) for the eight relationships (linear, quadratic, cubic, sine period 1/2, sine period 1/8, $x^{1/4}$, circle, and step), in three different settings (M_1, M_2, and M_3). The average of p-values below the significant level $\alpha = 0.05$ were calculated through 1,000 simulations.

		Simulation Model					
		M_1		M_2		M_3	
n	Relationship	GIMLET	MINDy	GIMLET	MINDy	GIMLET	MINDy
100	Line	**0.895**	0.576	0.621	0.090	0.652	0.141
	Quadratic	0.536	0.307	0.219	0.062	0.663	0.140
	Cubic	0.506	0.158	0.176	0.047	0.083	0.015
	Sine period 1/2	0.345	0.271	0.164	0.065	0.141	0.041
	Sine period 1/8	0.058	0.018	0.072	0.038	0.068	0.041
	$x^{1/4}$	0.750	0.314	0.241	0.055	0.708	0.200
	Circle	0.053	0.134	0.056	0.044	0.173	0.134
	Step	**0.880**	0.554	0.545	0.089	0.364	0.041
200	Line	**0.995**	**0.939**	**0.913**	0.121	**0.930**	0.315
	Quadratic	**0.861**	0.780	0.480	0.081	**0.935**	0.353
	Cubic	0.767	0.520	0.334	0.039	0.235	0.025
	Sine period 1/2	0.679	0.463	0.336	0.061	0.297	0.040
	Sine period 1/8	0.078	0.013	0.105	0.027	0.123	0.019
	$x^{1/4}$	**0.939**	0.693	0.474	0.043	**0.949**	0.405
	Circle	0.128	0.342	0.078	0.019	0.420	0.250
	Step	**0.994**	0.793	**0.866**	0.096	0.705	0.087
500	Line	**1.000**	**1.000**	**1.000**	0.208	**1.000**	0.761
	Quadratic	**0.995**	**1.000**	**0.885**	0.090	**1.000**	**0.822**
	Cubic	**0.981**	**0.979**	0.717	0.030	0.559	0.024
	Sine period 1/2	**0.934**	**0.997**	0.759	0.087	0.709	0.067
	Sine period 1/8	0.183	0.008	0.175	0.004	0.289	0.017
	$x^{1/4}$	**1.000**	**0.996**	**0.839**	0.035	**1.000**	**0.850**
	Circle	0.453	**0.905**	0.172	0.028	**0.916**	0.650
	Step	**1.000**	**0.998**	**0.997**	0.143	**0.965**	0.202

Table 1 shows the power calculated for eight different relationships with a varying sample size of 100, 200, and 500. Although both of the tested methods

have low power to detect modulators in small sample size (n=100), their power increases with the sample size. Note that GIMLET has higher power compared with MINDy in all relationships, except the circle. Both GIMLET and MINDy have low chances of identifying the modulators in the high-frequency sine relationship. MINDy was shown to outperform MINDy, especially in the settings M_2 and M_3, since MINDy is not designed as a multivariate dependence measure for the identification of modulators. Table 2 shows the type I error rates for the eight different relationships with a varying sample size of 100, 200, and 500. Type I error rates are quite close to the chosen α level for all the tests, demonstrating that GIMLET shows better type I error rate control, compared with MINDy, in this scenario.

Table 2. Type I error rate of GIMLET and MINDy, using synthetic data with different sample sizes ($n = 100, 200, 500$), for the eight relationships (linear, quadratic, cubic, sine period 1/2, sine period 1/8, $x^{1/4}$, circle, and step), in three different settings (M_1, M_2, and M_3). Type I error rate of a test should be equal to the significance level $\alpha = 0.05$.

		Simulation Model					
		M_1		M_2		M_3	
n	Relationship	GIMLET	MINDy	GIMLET	MINDy	GIMLET	MINDy
100	Line	**0.047**	**0.042**	**0.045**	0.071	**0.057**	0.061
	Quadratic	**0.041**	0.029	**0.052**	0.076	0.064	0.063
	Cubic	**0.050**	0.036	**0.051**	0.062	0.062	**0.051**
	Sine period 1/2	0.038	0.037	**0.058**	0.062	**0.053**	**0.053**
	Sine period 1/8	**0.045**	0.015	**0.041**	**0.051**	**0.057**	**0.040**
	$x^{1/4}$	**0.046**	0.023	**0.042**	0.061	**0.055**	**0.041**
	Circle	0.039	0.031	**0.056**	**0.055**	**0.049**	**0.056**
	Step	**0.053**	**0.049**	**0.048**	0.077	**0.054**	**0.056**
200	Line	**0.047**	0.035	**0.058**	0.066	**0.058**	0.034
	Quadratic	0.035	0.016	**0.052**	**0.059**	0.037	**0.044**
	Cubic	**0.045**	0.021	**0.048**	**0.046**	**0.043**	0.027
	Sine period 1/2	0.062	0.021	0.034	**0.047**	**0.043**	0.030
	Sine period 1/8	**0.049**	0.009	**0.045**	0.030	0.039	0.026
	$x^{1/4}$	**0.049**	0.030	**0.059**	**0.056**	**0.059**	**0.041**
	Circle	**0.059**	0.017	**0.048**	**0.056**	**0.046**	0.035
	Step	**0.046**	0.028	0.063	**0.058**	**0.055**	0.028
500	Line	**0.047**	0.021	**0.041**	0.030	**0.040**	0.012
	Quadratic	**0.051**	0.017	**0.053**	0.023	**0.046**	0.012
	Cubic	**0.046**	0.010	**0.048**	0.022	**0.047**	0.007
	Sine period 1/2	**0.060**	0.010	**0.053**	0.016	0.030	0.005
	Sine period 1/8	**0.053**	0.007	**0.053**	0.004	**0.045**	0.006
	$x^{1/4}$	**0.045**	0.018	**0.045**	0.024	**0.040**	0.013
	Circle	**0.056**	0.004	**0.049**	0.021	**0.053**	0.010
	Step	**0.046**	0.012	**0.044**	0.023	**0.047**	0.012

4 Results on real data

We first sought to identify genetic alterations that modulate the strength of the functional connection between HIF1A and the expression of its target genes in pan-kidney cohort in TCGA project [1]. The transcription factor HIF1A is a master transcriptional regulator of cellular and systemic homeostatic response to hypoxia by activating the transcription of genes that are involved in crucial aspects of cancer biology, including angiogenesis, cell survival, glucose metabolism and invasion, and is implicated in the development of clear cell renal clear cell carcinoma (ccRCC). We examined mRNA expression profiles from 536 ccRCC and 357 non-ccRCC (papillary RCC and chromophobe RCC) patients, somatic mutation profiles from 436 ccRCC and 348 non-ccRCC patients, and copy number profiles from 528 ccRCC and 354 non-ccRCC patients, which can be downloaded from the Broad GDAC Firehose website [17]. We used 90 literature-validated target genes of HIF1A from the Ingenuity Knowledge Base [18] and calculated the factor scores for each patient by performing maximum-likelihood single factor analysis on the expression data matrix of these genes. In this example, we considered the factor score as the unobserved activity of HIF1A at the protein level and used it as Y. As candidates of Z, we first tested somatic mutation of 85 genes, which were detected in more than 50 patients by genomic analyses of pan-kidney cohort. We next considered copy-number alterations of 41 chromosomal arms as candidates of Z. For this analysis, we would expect to find alteration of von Hippel-Lindau (VHL) tumor suppressor gene, which leads to overexpression of HIF1A and is a critical event in the pathogenesis of most ccRCC [19].

Table 3. Five significantly associated gene mutations and genetic alterations modulating HIF1A activity.

modulator	type	q-value	ldcor (no mut/alt)	ldcor (mut/alt)
VHL	Mutation	0.001	0.24	0.49
3p	Deletion	0.001	0.23	0.44
20q	Amplification	0.001	0.42	0.20
20p	Amplification	0.002	0.42	0.20
PBRM1	Mutation	0.006	0.27	0.51

The modulator analysis of GIMLET yields five significantly associated gene mutations and genetic alterations modulating HIF1A activity with q-value<0.10 (Table 3). Indeed, GIMLET identified VHL as the most significantly associated gene mutation. Although PBRM1, identified as the second-most significantly associated gene mutation, is not reported to directly modulate HIF1A activity, this result remains significant since almost all PBRM1 mutant cases also have dysregulation of the hypoxia signaling pathway [20] and it is likely that PBRM1 and VHL cooperate in kidney carcinogenesis leading to overexpression of hypoxia-inducible transcription of HIF1A. The analysis also yields three regions significantly modulating HIF1A activity with q-value<0.10. Chromosome

3p deletions are observed in approximately 90% of ccRCC, which harbors VHL and tumor suppressor genes [21].

We next examined drug-treated gene expression profiles from Broad Institute The Library of Integrated Cellular Signatures (LINCS) Center for Transcriptomics [22]. We sought to use these data to identify drugs that inhibit the strength of the functional connection between FOXM1 and CENPF which are master regulators of prostate cancer malignancy [23] and the expression of their target genes. A total of perturbational gene expression profiles of 22,268 probes for 6,684 experiments treated with 271 compounds after 24 hours under different doses (0.04, 0.12, 0.37, 1.11, 3.33, and 10 um) in the two prostate cancer cell lines, PC3 and LNCaP, has been downloaded from the LINCS L1000 dataset [22]. The expression values for each profile were normalized by robust z-scores relative to control (plate population) and summarized using the median across replicates. If there are multiple probes which correspond to the same gene, the probe with the highest variance across all samples was selected as a single representative probe. Finally, the expression matrix data of 12,716 genes and 1,976 samples were used for further analysis. We used the expression of FOXM1 and CENPF as X and their unobserved activity as Y which was defined using maximum-likelihood single factor analysis on the expression data matrix for the 173 and 55 literature-validated targets of FOXM1 and CENPF from the Ingenuity Knowledge Base [18]. Drug target genes for each compound under a given dose level were defined as differentially expressed genes which were significantly lower in drug-treated cell lines than in vehicle-treated cell lines using one-tailed t-test (p-value$<$0.001). As candidates of Z, the drug-perturbational activity for each sample under each of 1850 different pertubagens was then estimated using enrichment scores (maxmean statistics) of these drug target gene sets for Gene Set Analysis [24]. We applied GIMLET to identify functional pertubagens modulating FOXM1 and CENPF activity.

Table 4. Thirteen significantly associated modulators (pertubagens) modulating FOXM1 and CENPF activity.

modulator	dose	cell line	target	-$\log_{10}$(q-value)
Vorinostat	10um	PC3	HDAC1	9.91
Withaferin A	3.33um	PC3	MMP2	9.63
Dasatinib	0.37um	PC3	ABL1	9.02
Dasatinib	0.12um	PC3	ABL1	8.38
JW-7-24-1	10um	PC3	LCK	8.38
OSI-027	10um	PC3	mTOR	8.38
Radicicol	10um	PC3	HSP90	8.38
PHA-793887	3.33um	LNCaP	CDK2	8.30
WYE-125132	10um	PC3	mTOR	8.07
GSK-1059615	0.37um	PC3	PI3K	7.39
Sirolimus	0.37um	LNCaP	mTOR	7.38
WYE-125132	10um	PC3	mTOR	7.38
Celastrol	1.11um	LNCaP	PSB5	7.20

The analysis yields 13 pertubagens which significantly inhibit the regulation of FOXM1 and CENPF with global q-value$< 10^{-7}$ (Table 4). Indeed, these pertubagens support inhibition of tumor progression in human prostate cancer by several resent studies. For example, Vorinostat known as suberanilohydroxamic acid is a member of a larger class of compounds that inhibit histone deacetylases (HDAC) [25]. The past study has also shown that Vorinostat may inhibit tumor growth by both oral and parenteral administration in prostate cancer [26]. Withaferin A, a major bioactive component of the Indian herb Withania somnifera, induces cell death and inhibits tumor growth in human prostate cancer [27]. Activation of the PI3K-AKT-mTOR pathway is extremely common, if not universal, in castrate-resistant prostate cancer [28]. Some PI3K and mTOR inhibitors are currently under investigation in clinical trials for CRPC including the dual inhibitor NVP-BEZ235 [29], and the mTOR inhibitor RAD001 or everolimus [30,31].

The analyses with two examples thus show that GIMLET can identify genetic alterations and functional pertubagens modulating the relationship between a given set of regulators and the expression of their target genes in particular cancer subtypes.

5 Discussion

The identification of modulators is a challenging problem for the researchers who study gene regulation. The paradigm introduced by [4] and the state-of-the-art classical methods for the identification of modulators are quite useful because they allow us to identify the content-specific modulators of a transcription factor activity using gene expression data. However, these methods are restricted to the capturing of a particular type of dependency between univariate random variables, and it can be difficult to describe more complex multivariate dependency structures, where multiple transcription factors and modulators are functionally related. We have developed a more general class of the identification of modulators, in the framework of energy statistics and a specific implementation, called GIMLET. An appealing property of the proposed method is that it can measure all types of dependency, including non-monotonic and non-linear relationships, between random vectors in an arbitrary dimension easily. Our simulation results demonstrate that GIMLET outperforms MINDy in terms of statistical power and type I error rate. The analysis with a real example thus shows that GIMLET can identify genetic alterations and functional pertubagens modulating transcription factor activities. We believe that the presented method may be useful for a range of biological applications, and it can represent a breakthrough in gene regulation research.

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