RoDiCE: Robust differential protein co-expression analysis for cancer complexome

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Abstract

Motivation: The full picture of abnormalities in protein complexes in cancer remains largely unknown. Comparing the co-expression structure of each protein complex between tumor and normal groups could help us understand the cancer-specific dysfunction of proteins. However, the technical limitations of mass spectrometry-based proteomics and biological variations contaminating the protein expression with noise lead to non-negligible over- (or under-) estimating co-expression.

Results: We propose a robust algorithm for identifying protein complex aberrations in cancer based on differential protein co-expression testing. Our method based on a copula is sufficient for improving the identification accuracy with noisy data over a conventional linear correlation-based approach. As an application, we show that important protein complexes can be identified along with regulatory signaling pathways, and even drug targets can be identified using large-scale proteomics data from renal cancer. The proposed approach goes beyond traditional linear correlations to provide insights into higher order differential co-expression structures.

Availability and Implementation: <https://github.com/ymatts/RoDiCE>.

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Supplementary information: Supplementary data are available online.

1 Introduction

Cancer is a complex system. Many molecular events, such as genomic mutations and epigenetic and transcriptomic dysregulations, were identified as cancer drivers (Hoadley *et al.*, 2018). However, our knowledge of how they characterize the downstream mechanisms with proteomic phenotypes remains scarce (Clark *et al.*, 2019; Liu *et al.*, 2016; Mertins *et al.*, 2016; Zhang *et al.*, 2016). Protein complexes are responsible for most cellular activities. Recent studies (Ori *et al.*, 2016; Romanov *et al.*, 2019; Ryan *et al.*, 2017) have demonstrated that protein subunits tend to show co-expression patterns in proteome profiles; furthermore, the subunits of a complex are

simultaneously down-/up-regulated with the genomic mutations (Ryan *et al.*, 2017). However, we know little about the changes in the co-regulatory modes of protein complexes between the tumor and normal tissues.

We propose a novel algorithm for differential co-expression of protein abundances to identify the tumor-specific abnormality of protein complexes. Differential co-expression (DC) analysis is a standard technique of gene expression analysis to find differential modes of co-regulation between conditions, and numerous methods already exist (Bhuva *et al.*, 2019). Correlation is one of the most common measures of co-expression. For example, differential correlation analysis (DiffCorr) (Fukushima, 2013) and gene set co-expression analysis (GSCA) (Choi and Kendzierski, 2009) are two-sample tests of Pearson's correlation coefficients. However, studies report that protein expression levels have greater variability than gene expression levels because of the regulatory mechanism of post-translational modifications (Gunawardana *et al.*, 2015; Liu *et al.*, 2016). This variability can affect the estimation of co-expression as an outlier and can significantly impact DC results.

We developed a robust DC framework, called Robust Differential Co-Expression Analysis (RoDiCE), via two-sample randomization tests with empirical copula. The notable advantage of RoDiCE is noise robustness. Our main contributions are as follows: 1) we develop an efficient algorithm for robust copula-based statistical DC testing; 2) we overcome the computational hurdles of the copula-based permutation test by incorporating extreme value theory; 3) we demonstrate the effective application of copula to cancer complexome analysis; and 4) we develop a computationally efficient multi-thread implementing as R package.

1.1 Motivational example from the CPTAC / TCGA dataset

First, using an actual dataset, we explain why there is a need for robustness in protein co-expression analysis. We analyzed a cancer proteome dataset of clear renal cell carcinoma from CPTAC/TCGA with 110 tumor tissue samples. We measured co-expression using Pearson's correlation coefficient. We compared the correlation coefficients before and after removing the outliers. To identify outlier samples, we applied robust principal component analysis using the R package ROBPCA (Hubert *et al.*, 2005) with default parameters. Among 49,635,666 pairs of 9,964 proteins, the correlation coefficients of 7,541,853 (15.2%) pairs were deviated by more than 0.2 after removing outlier samples (**Figure 1**). This result implied that a non-negligible proportion of protein co-expression would be overestimated or underestimated. To compare the structures of co-expression correctly, it is necessary to compare them while minimizing the over-/under-estimation of co-expression.

2 Methods

Figure 2 describes the outline of RoDiCE. We decompose the expression level of subunits in the protein complex into a structure representing a co-expression and one representing the expression level of each subunit, using a function called an empirical copula (Nelsen, 2010); the empirical copula rank-converts the scale of the original data. Comparing the empirical copula functions with the conditions of statistical hypothesis testing, we derive the p -value as the difference in co-expression structures. We describe our method in detail in the following sections.

2.1 RoDiCE model

Suppose there are n samples, and $g(g = g_1, g_2)$ represents each condition. We compare two conditions and assume that g_1 and g_2 represent the normal group and the tumor group, respectively. Let $\mathbf{X}_g = (X_{1g}, X_{2g}, \dots, X_{Pg})$ be abundances of P subunits in group g . Given a protein complex, we represent the entire behaviors of subunits with a joint distribution $\mathbf{X}_g \sim H_g(x_1, x_2, \dots, x_P)$. The distribution function H_g has two pieces of information as follows: subunit expression levels and the structure of co-expression between subunits. The copula C_g is a function that can decompose those two pieces of information into a form that can be handled separately, as follows:

$$H_g(x_1, x_2, \dots, x_P) = C_g(F_{1g}(x_1), F_{2g}(x_2), \dots, F_{Pg}(x_P)) \quad (1)$$

The behavior of each subunit $F_{pg}(x_p)$ is represented by a distribution function. The copula function itself is a multivariate distribution with uniform marginals. The copula function includes all dependency information among the subunits (Nelsen, 2010; Rémillard and Scaillet, 2009; Seo, 2020).

We use the empirical copula to non-parametrically estimate the copula C_g since it could be widely applicable to various situations. It can be represented using pseudo-copula samples defined via rank-transformed subunit abundance $u_{ip} = \frac{R(x_{ip})}{n} (i = 1, 2, \dots, n)$;

$$\hat{C}_g(u_1, u_2, \dots, u_p) = \frac{1}{n} \sum_i I(U_{1g} \leq u_1, U_{2g} \leq u_2, \dots, U_{pg} \leq u_p) \quad (2)$$

where $R(\cdot)$ is a rank-transform function, and we represent transformed pseudo-sample variables as $R(\mathbf{X}_{g_1}) = \mathbf{U}_{g_1}$ and $R(\mathbf{X}_{g_2}) = \mathbf{U}_{g_2}$. The empirical copula is robust to noise because it represents co-expression structures based on rank-transformed subunit expression levels, which is the so called scale invariant property in the context of copula theory (Nelsen, 2010).

To perform DC analysis between group g and g' , we consider the following statistical hypothesis:

$$\begin{aligned} \mathcal{H}_0: C_{g_1} &= C_{g_2} \\ \mathcal{H}_1: C_{g_1} &\neq C_{g_2} \end{aligned} \quad (3)$$

1 We derive the following Cramér-von Mises type test statistic to perform statistical hypothesis
2 testing (Rémillard and Scaillet, 2009):

$$\begin{aligned} s(g, g') = & \left(\frac{1}{n_{g_1} + n_{g_2}} \right)^{-1} \left\{ \frac{1}{n_1^2} \sum_{i=1}^{n_{g_1}} \sum_{j=1}^{n_{g_1}} \prod_{p=1}^P \max \left(1 - u_{ip}^{(g_1)}, u_{jp}^{(g_1)} \right) \right. \\ & - \frac{2}{n_{g_1} n_{g_2}} \sum_{i=1}^{n_{g_1}} \sum_{j=1}^{n_{g_2}} \prod_{p=1}^P \max \left(1 - u_{ip}^{(g_1)}, u_{jp}^{(g_2)} \right) \\ & \left. + \frac{1}{n_{g_2}^2} \sum_{i=1}^{n_{g_2}} \sum_{j=1}^{n_{g_2}} \prod_{p=1}^P \max \left(1 - u_{ip}^{(g_2)}, u_{jp}^{(g_2)} \right) \right\} \end{aligned} \quad (4)$$

4 where $u_{ip}^{(g)} (i = 1, 2, \dots, n_g)$ represents pseudo-observation in group g . Note that the
5 computational cost is n^2 , where $n^2 \leq n_{g_1} n_{g_2}$; $n = \min(n_{g_1}, n_{g_2})$. For testing the test statistic
6 (4), we also derived the p -value using an algorithm based on Monte Carlo calculations
7 (Rémillard and Scaillet, 2009); however, the computational complexity of the algorithm makes it
8 difficult to apply it to proteome-wide co-expression differential analysis (see the results of the
9 simulation experiments described below).

10 2.2 Derivation of statistical significance

11 Using a permutation test, we derive the p -value using the following steps:

- 12 (1) Randomizing concatenated variable from the two groups; $\mathbf{W} = (\mathbf{U}_{g_1}, \mathbf{U}_{g_2})$
- 13 (2) Constructing a new randomized variable $\mathbf{U}'_{g_1} = (W_{r(1)}, W_{r(2)}, \dots, W_{r(n_1)})$ and $\mathbf{U}'_{g_2} =$
14 $(W_{r(n_1+1)}, W_{r(n_1+2)}, \dots, W_{r(n_1+n_2)})$ with randomized index $r(i)$.
- 15 (3) Replacing copula functions C_{g_1} and C_{g_2} in (3) with re-estimated empirical copula
16 function C'_{g_1} and C'_{g_2} from the randomized samples $\mathbf{X}'_{g_1}$ and $\mathbf{X}'_{g_2}$.
- 17 (4) Deriving test statistics $s'(g_1, g_2)$ based on (4) with C'_{g_1} and C'_{g_2} .
- 18 (5) Steps 2 and 3 are indispensable for deriving the null distribution correctly. Deriving the
19 null distribution by randomizing $\mathbf{W}' = (\mathbf{U}_{g_1}, \mathbf{U}_{g_2})$ alone will distort the distribution, and
20 we will be unable to control for the type I error correctly (Seo, 2020).

21 2.3 Approximation of p -value

22 The empirical p -value is derived as follows:

$$p(M) = 1 - \frac{\sum_{i=1}^M \mathbf{I}(S_i \leq s_{g,g'})}{M} \quad (5)$$

24 where M is the number of randomization and S_i is the test statistic from the null distribution
25 of the i -th ($i = 1, 2, \dots, M$) randomization trials. The accuracy of p -value in (5) is bounded by
26 $p(M) \geq 1/M$. As mentioned, calculating the test statistic requires a computational cost of

1 $O(n^2)$; therefore, an efficient computational algorithm is needed to derive accurate p -values in
 2 data with a large number of samples. For instance, proteomic cohort projects such as CPTAC /
 3 TCGA have more than $n = 100$ samples. To address this problem, we introduced an
 4 approximation algorithm for p -values based on extreme value theory (Knijnenburg *et al.*, 2009)
 5 and devised a way to calculate accurate p -values even with a small number of trials.

6 The test statistic that exceeds the range of the accuracy with randomization trials M is regarded
 7 as an “extreme value,” and its tail of the distribution could be estimated via a generalized Pareto
 8 distribution (GPD), as follows:

$$9 \quad p_{approx} = \frac{N'}{N} (1 - G(s(g, g') - t)) \quad (6)$$

10 where N' is the number of the randomized test statistic exceeding the threshold t that has to be
 11 estimated via a goodness-of-fit (GoF) test (Knijnenburg *et al.*, 2009) and G is the cumulative
 12 distribution function of the generalized Pareto distribution, $G(x) = 1 - \left(1 - \frac{kx}{a}\right)^{\frac{1}{k}}$ for $k \neq$
 13 0 and $G(x) = 1 - e^{-\frac{x}{a}}$ for $k = 0$. To estimate the threshold t in (6), the GoF test determines
 14 whether the excess comes from the distribution $G(x)$ via bootstrap based maximum likelihood
 15 estimator (Villaseñor-Alva and González-Estrada, 2009). As we do not know a priori the number
 16 of samples sufficient to estimate the underlying GPD with threshold t , we must decide the initial
 17 number of samples to use. We begin with a large number of samples and increase this number
 18 until the GoF test is not rejected, according to (Knijnenburg *et al.*, 2009). As initial samples, we
 19 start with those above 80% of quantiles and decrease samples by 1% while the GoF test is rejected.

20 **2.4 Identification of protein complex alteration**

21 As protein complexes show co-expression among multiple subunits (Kerrigan *et al.*, 2011), we
 22 hypothesized that the difference in the co-expression structure of the tumor group compared to
 23 the normal group is a characteristic quantity of the protein complex abnormality. In previous
 24 studies of the cancer transcriptome, differential co-expression analysis has revealed abnormalities
 25 associated with protein complexes (Amar *et al.*, 2013; Srihari *et al.*, 2014). Therefore, we define
 26 a protein complex as an abnormal protein complex when it is co-expressed in at least one pair of
 27 subunits. Thus, we applied RoDiCE to all protein complexes for each subunit pair ($p = 2$) and
 28 identified protein complexes that showed a statistically significant difference in at least one
 29 subunit pair as abnormal.

30 **2.5 Protein membership with protein complex**

31 As we do not know which proteins belong to which protein complexes, we must predict the
 32 membership via some method. There are two main approaches. One is membership prediction

1 focusing on the modular structure in PPI networks (Adamcsek *et al.*, 2006; Nepusz *et al.*, 2012)
2 and the other is a knowledge-based method using a curated database. We adopt the latter
3 approach, which is based on already validated protein complex membership information, using
4 CORUM (ver. 3.0)(Giurgiu *et al.*, 2019) as a database (see the Supplementary Data for details).

5 **2.6 R implementation with multi-thread parallelization**

6 To further accelerate the computation of test statistic (4) in the randomization steps, we used
7 RcppParallel (Allaire J, 2019). We utilize the portable and high-level parallel function
8 “parallelFor,” which uses Intel TBB of the C++ library as a backend on systems that support it
9 and TinyThread on other platforms.

10 **2.7 Copula-based simulation model for protein co-expression**

11 We provide the outline of a method for simulating co-expressed structures using a copula. We
12 simulated protein expression levels that showed differential co-expression patterns with outliers
13 in the tumor group and the normal group. We represented the co-expression structure by the
14 covariance parameter in the following bivariate Gaussian copula:

$$15 \quad C_g(u_1, u_2) = \Phi_g \left(\phi^{-1}(x_1), \phi^{-1}(x_2); \Sigma_{p=2}^{(g)} \right) \quad (7)$$

16 where Φ_g is the p dimensional Gaussian distribution parameterized by a
17 $p \times p$ covariance matrix (or correlation matrix) in the group g , denoted as $\Sigma_p^{(g)} = \{r_{ij}^{(g)}\}$ and
18 $\phi(x_i)$ is a univariate distribution. Using the model, we generate the dependency structure with
19 two groups; one group has high correlations and the other has low ones, $r_{ij}^{(g_1)} \sim U(0.8, 0.9)$ and
20 $r_{ij}^{(g_2)} \sim U(0.1, 0.2)$, respectively. We then generated co-expression structure using a Gaussian
21 copula with $\phi(x) = N(0, 1)$. We obtained protein expressions via

$$22 \quad H_g(x_1, x_2) = C_g \left(F_{1g}(x_1), F_{2g}(x_2) \right) \quad (8)$$

23 where we simply set as $F_{ig} \sim N(\mu, \sigma)$ for $i = 1, 2$ and $g = g_1, g_2$ with $\mu \sim N(2, 1)$ and
24 $\sigma \sim \text{gamma}(2, 1)$. Furthermore, we added outliers that could affect the co-expression structure.
25 Using the model in (6) and (7), we set the outlier population in both group as
26 $r'_{ij}^{(g_1)} \sim U(0, 0.05)$ and $F'_{ig} \sim N(2, 4)$ for $i = 1, 2$ and $g = g_1, g_2$ (Fig3).

27 **3 Results**

3.1 Benchmarking RoDiCE with simulation dataset

We now describe the features of RoDiCE using a simulation model. First, to confirm whether RoDiCE could correctly derive the p -value, we performed a test on two groups, with no differences in co-expression structure without outliers, and confirmed the null rejection rate. We performed 100 tests with the proposed method and calculated the null rejection rate at the 1%, 5%, and 10% levels of significance. The same simulation was repeated 10 times to calculate the standard deviations. The results show that the proposed method can control type I errors (Table 1).

Table 1. Type I error controls of the proposed method

Significance Level	Mean	SD
1%	0.03	0.02
5%	0.05	0.02
10%	0.09	0.02

We then simulated a case in which the co-expressed structure between the two groups was different and included outliers, and we examined the sensitivity of the method to identify a broken co-expressed structure in tumor tissue relative to normal tissue. To demonstrate the advantages of the proposed method, we examined the sensitivity of increasing the percentage of outliers in 2% increments from 0% to 20% and compared it further with DiffCorr and GSCA, a two-group co-expression test method based on Pearson's linear correlation (**Figure 4**). For outliers, the proposed method showed robust co-expression test results, with an accuracy of more than 85% up to a percentage of outliers of approximately 15%. Conversely, the sensitivity of the method based on linear correlation starts to decline from the level of 2% of outliers, and for data containing 15% of outliers, the sensitivity drops to around 30%.

To investigate the relationship between sample size and identification accuracy, we simulated the sensitivity of RoDiCE, as we increased the number of samples in increments of 10 from 30 to 100 samples. All other settings were the same as those in **Figure 5**, except that the percentage of outliers was set at 5%.

Finally, we also examined the computational speed, comparing it with the R package TwoCop, which implements the Monte Carlo-based method (ref) used for the two-group comparison of copulas (**Table 2**). The proposed method is 68 times faster than TwoCop and is sufficiently efficient as a copula-based two-group comparison test method. In contrast, the estimation of the

1 copula function required more computational time than the linear correlation coefficient-based
2 method because of the computational complexity of estimating the copula function.

3 **Table 2.** Computation time for 10 replicates

Method	#Replications	Execution time (s)	Relative time
DiffCorr	10	0.001	1
GSCA	10	0.152	152
RoDiCE	10	0.373	373
TwoCop	10	25.301	25301

4 **3.2 Application to cancer complexome analysis**

5 We demonstrate RoDiCE with actual data using the clear renal cell carcinoma (ccRCC)
6 published by CPTAC/TCGA (Clark *et al.*, 2019). The data are available from the CPTAC data
7 portal (<https://cptac-data-portal.georgetown.edu>) in the CPTAC Clear Cell Renal Cell Carcinoma
8 (CCRCC) discovery study. The data labeled
9 "CPTAC_CompRef_CCRCC_Proteome_CDAP_Protein_Report.r1" were used. In the following
10 analysis, only protein expression data that overlap with protein groups in human protein
11 complexes in CORUM and in CPTAC were used. Missing values were completed based on
12 principal component analysis, and the missing values were completed by 10 principal components
13 using the `pca` function in `pca` Methods.

14 For the complete data, RoDiCE was applied to the normal and cancer groups for each protein
15 complex. FDR was calculated by correcting the p-value for each complex using the Benjamini–
16 Hochberg method. We identified anomalous protein complexes in protein expression data from
17 110 tumor and 84 normal samples; out of 3,364 protein complexes in CORUM, 1,244 complexes
18 contained at least one co-expression difference between subunits with $FDR \leq 5\%$
19 (**Supplementary Data**).

20 The proposed method has identified several protein complexes containing driver genes on
21 regulatory signaling pathways in ccRCC (**Figure 6a**) (Li *et al.*, 2019). The identified pathways
22 included known regulatory pathways important for cancer establishment and progression, starting
23 with chromosome 3p loss, regulation of the cellular oxygen environment (VHL), chromatin
24 remodeling, and disruption of DNA methylation mechanisms (PBRM1, BAP1). They also
25 included abnormalities in regulatory signals involved in cancer progression (AKT1). Moreover,
26 several identified complexes also included key proteins, for example, MET, HGF, and FGFR
27 proteins, which could be inhibited by targeting them with drugs such as Cabozantinib and
28 Lenvatinib directly. Because a previous study reported that sensitivity to knockdowns of several

genes was well associated with expression levels of protein complexes (Nusinow *et al.*, 2020), co-expression information on protein complexes containing druggable genes might be useful to optimize drug selection.

A close examination of the above identified protein complexes allows us to partially understand how the dysregulation of protein was a co-expression abnormality between VHL and TBP1. The upregulation of TBP1 is known to induce dysregulation of downstream HIF1A molecules in a VHL-dependent manner (Corn *et al.*, 2003). In fact, the protein expression of TBP1 increased in the tumor group. We also examined the PBAF complex containing the driver gene PBRM1, which is thought to occur following VHL abnormalities. Along with a decrease in PBRM1 protein expression, there was a loss of tumor group-specific co-expression structure among many subunits involved with PBRM1 levels.

4 Discussion

In this study, we developed an algorithm of robust identification for protein complex aberrations based on differential co-expression structure using protein abundance. Protein expression data measured through LC/MS/MS contains a non-negligible percentage of outliers due to technical limitations and variation due to biological reasons such as post-translational modifications. This causes the problem of over- (or under-) estimation of co-expression. The copula-based DC approach is a powerful statistical framework as a solution to this problem.

In addition to noise robustness, this study does not include several other key properties of the copula that are important in capturing the co-expression structure. The first is self-equitability (Chang *et al.*, 2016; Ding *et al.*, 2017). Copulas can capture nonlinear structures between variables, and self-equitability allows us to evaluate the degree of dependency equally between variables in linear and nonlinear relations. Therefore, copula allows us to compare a much broader range of co-expressed structures than conventional linear and nonlinear correlations.

Second, we can also model simultaneous co-expression structures between three or more proteins. Although this study only identified pairwise co-expression differences, equation (4) allows the identification of simultaneous co-expression differences across three or more proteins. However, high-dimensional estimation of the copula remains limited, and at present, in our simulations, the comparison of simultaneous co-expressed structures of 15 proteins is a performance limitation for about 100 samples.

As described, the copula-based co-expression analysis approach is a powerful modeling method for data sets where noise is expected, although there remain challenges in high-dimensional estimation. In particular, it could be useful for modeling proteome-wide protein expression patterns. The proposed approach is useful for understanding the abnormalities in the protein

complexes of cancer. Studies focusing on protein complexes in large-scale cancer proteomics are in their infancy. We believe that this approach will provide valuable insights into the molecular mechanisms of cancer and the search for new drug targets.

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Conflict of Interest: none declared.

Data Availability Statement

The data underlying this article are available in CPTAC data portal, and CORUM3.0. The datasets were derived from sources in the public domain: <https://cptac-data-portal.georgetown.edu> and <http://mips.helmholtz-muenchen.de/corum/>.

References

- Adamcsek, B. et al. (2006) CFinder: locating cliques and overlapping modules in biological networks. *Bioinformatics*, 22(8), 1021-1023.
- Allaire J., Francois, R., Ushey, K., Vandenbrouck, G., Geelnard, M. Intel (2019) RcppParallel: Parallel Programming Tools for 'Rcpp'. R package version 4.4.4.
- Amar, D. et al. (2013) Dissection of regulatory networks that are altered in disease via differential co-expression. *PLoS Comput Biol*, 9(3), e1002955.
- Bhuva, D.D. et al. (2019) Differential co-expression-based detection of conditional relationships in transcriptional data: comparative analysis and application to breast cancer. *Genome Biol*, 20(1), 236.
- Chang, Y. et al. A robust-equitable copula dependence measure for feature selection. In: Arthur, G. and Christian, C.R., editors, *Proceedings of the 19th International Conference on Artificial Intelligence and Statistics*. Proceedings of Machine Learning Research: PMLR; 2016. p. 84-92.
- Choi, Y. and Kendzierski, C. (2009) Statistical methods for gene set co-expression analysis.

Bioinformatics, 25(21), 2780-2786.

Clark, D.J. et al. (2019) Integrated proteogenomic characterization of clear cell renal cell carcinoma. *Cell*, 179(4), 964-983 e931.

Corn, P.G. et al. (2003) Tat-binding protein-1, a component of the 26S proteasome, contributes to the E3 ubiquitin ligase function of the von Hippel–Lindau protein. *Nat Genet*, 35(3), 229-237.

Ding, A.A. et al. (2017) A robust-equitable measure for feature ranking and selection. *J Mach Learn Res*, 18(1), 2394-2439.

Fukushima, A. (2013) DiffCorr: an R package to analyze and visualize differential correlations in biological networks. *Gene*, 518(1), 209-214.

Giurgiu, M. et al. (2019) CORUM: the comprehensive resource of mammalian protein complexes-2019. *Nucleic Acids Res*, 47(D1), D559-D563.

Gunawardana, Y. et al. (2015) Outlier detection at the transcriptome-proteome interface. *Bioinformatics*, 31(15), 2530-2536.

Hoadley, K.A. et al. (2018) Cell-of-origin patterns dominate the molecular classification of 10,000 tumors from 33 types of cancer. *Cell*, 173(2), 291-304.e296.

Hubert, M. et al. (2005) ROBPCA: A new approach to robust principal component analysis. *Technometrics*, 47(1), 64-79.

Kerrigan, J.J. et al. (2011) Production of protein complexes via co-expression. *Protein Expr Purif*, 75(1), 1-14.

Knijnenburg, T.A. et al. (2009) Fewer permutations, more accurate P-values. *Bioinformatics* (Oxford, England), 25(12), i161-i168.

Li, Q.K. et al. (2019) Challenges and opportunities in the proteomic characterization of clear cell renal cell carcinoma (ccRCC): A critical step towards the personalized care of renal cancers. *Semin Cancer Biol*, 55, 8-15.

Liu, Y. et al. (2016) On the dependency of cellular protein levels on mrna abundance. *Cell*, 165(3), 535-550.

Mertins, P. et al. (2016) Proteogenomics connects somatic mutations to signalling in breast cancer. *Nature*, 534(7605), 55-62.

Nelsen, R.B. An introduction to copulas. Springer Publishing Company, Incorporated; 2010.

Nepusz, T. et al. (2012) Detecting overlapping protein complexes in protein-protein interaction networks. *Nat Methods*, 9(5), 471-472.

Nusinow, D.P. et al. (2020) Quantitative proteomics of the cancer cell line encyclopedia. *Cell*, 180(2), 387-402.e316.

Ori, A. et al. (2016) Spatiotemporal variation of mammalian protein complex stoichiometries. *Genome Biol*, 17(1), 47.

Rémillard, B. and Scaillet, O. (2009) Testing for equality between two copulas. *J Multivar Anal*,

1 100(3), 377-386.
2 Romanov, N. et al. (2019) Disentangling genetic and environmental effects on the proteotypes of
3 individuals. *Cell*, 177(5), 1308-1318.e1310.
4 Ryan, C.J. et al. (2017) A compendium of co-regulated protein complexes in breast cancer reveals
5 collateral loss events. *Cell Syst*, 5(4), 399-409 e395.
6 Seo, J. (2020) Randomization tests for equality in dependence structure. *J Bus Econ Stat*, 1-35.
7 Srihari, S. et al. (2014) Complex-based analysis of dysregulated cellular processes in cancer.
8 *BMC Syst Biol*, 8(4), S1.
9 Villaseñor-Alva, J.A. and González-Estrada, E. (2009) A bootstrap goodness of fit test for the
10 generalized Pareto distribution. *Comput Stat Data Anal*, 53(11), 3835-3841.
11 Zhang, H. et al. (2016) Integrated proteogenomic characterization of human high-grade serous
12 ovarian cancer. *Cell*, 166(3), 755-765.
13

1

2 **Fig 1. Actual example of effects of outliers on co-expression.** Difference in Pearson's correlation
3 before and after removing outlier samples; the left panel shows a histogram of the difference in
4 correlation differences. The right panel shows a scatter plot of the original correlation against one without
5 outlier samples.

6

7 **Fig 2. Overview of RoDiCE. a) Objective of the analysis via RoDiCE.** The proposed method aims to
8 identify abnormal protein complexes by comparing two abnormal groups. An abnormal complex is one
9 where the co-expressed structure is different in at least two subunits. **b) Protein co-expression and outliers.**
10 The protein expression levels measured through LC/MS/MS contain some outliers because of the addition
11 of noise from several sources. These can cause over- (or under-) estimation in the co-expression structure.
12 **c) Copula decomposition.** The RoDiCE model decomposes the observed joint distributions of protein
13 expression into a marginal distribution representing the behavior of each protein and an empirical copula
14 function representing the latent co-expression structures between proteins. This allows us to extract
15 potential co-expressed structures and compare them robustly against outliers. The figure shows an example
16 where the co-expressed structure estimated by copula is actually the same for two apparently different joint
17 distributions of protein expression. **d) Copula robustness.** A copula is a function that expresses a
18 dependency on a rank-transformed space of data scales. One advantage of transforming the original scale
19 into a space of rank scale is that it is robust to outliers. The example in the figure compares Pearson's linear
20 correlations with Pearson's linear correlations in the space converted to a rank scale by a copula function
21 (Spearman's linear correlations). Pearson's linear correlation underestimates from 0.74 to 0.44 due to
22 outliers, whereas the linear correlation on the rank scale has a relatively small effect (0.72 to 0.62). **e)**
23 **RoDiCE is a copula-based two-sample test.** RoDiCE is an efficient method for testing differences in
24 copula functions between two groups. Rather than a summary measure such as correlation coefficients, we
25 compare copula functions expressing overall dependence between groups. This allows us to robustly
26 identify differences in complex co-expression structures between two groups of protein complexes to
27 outliers.

28

29

30 **Fig 3. Simulated dataset.** Generated samples in the numerical experiments for the bivariate case.
31 To mimic the noises in proteome abundance dataset, the outlier population was assumed other than
32 the that of tumor and normal population.

33

34

1 **Fig 4. Sensitivities and ratio of outliers.** The percentage of outliers is taken on the horizontal axis,
2 and the sensitivity of the co-expression differences by each method (5% level of significance) is
3 shown on the vertical axis.

4
5
6 **Fig 5. Sensitivities and sample size.** The horizontal axis shows the sample size, while the
7 vertical axis shows the sensitivity of the co-expression differences by each method (5% level of
8 significance).

9
10 **Fig 6. Identified protein complexome related to driver genes. a) Dysregulated protein**
11 **complex with known driver and druggable genes.** The red shows the pairs with differential
12 co-expression between the subunits of the protein complexes (5% level of significance). The
13 thickness of the line is proportional to $-\log_{10}(\text{p-value})$. The blue lines are the non-significant
14 pairs. The yellow nodes represent proteins whose expression was actually measured by
15 LC/MS/MS in this study, and the gray ones represent proteins that were not measured. **b)**
16 **Examples of VHL- TBP1-HIF1A complex and PBAF complex with the co-expression**
17 **structure.** Blue and red represent the tumor and normal groups, respectively, and the density
18 distribution of protein expression is shown on the diagonal. In the lower diagonal, the co-
19 expression pattern before copula-transformations is illustrated. The co-expression pattern after
20 copula-transformations is illustrated in the upper diagonal.

21

AKT1-APPL1-HDAC3 complex (AKT)

FGF1-FGFR1-ITGB3 complex (FGFR)

PBAF complex (PBRM1)

**VEGFR2-S1PR1-ERK1_2-
PKC-alpha complex (VEGFR)**

CEN complex (BAP1)

MET-CIN85-SH3GL3-CBL complex (MET)

ARAF-PIK3R1 complex (PIK3R1)

VHL-TBP1-HIF1A complex (VHL)

PTEN-NHERF1-EGFR complex (PTEN)

EGFR-containing signaling complex (EGFR)

a

VHL-TBP1-HIF1A complex (VHL)

b

PBAF complex (PBRM1)