NetWeAvers: an R package for integrative biological network analysis with mass spectrometry data

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1 INTRODUCTION

The statistical analysis of protein–protein interaction networks (PPINs) in conjunction with mass spectrometry (MS) data is an important step in a proteomics analysis pipeline. Here we describe NetWeAvers (Network Weighted Averages) for analyzing groups of regulated proteins in a network context, e.g. as defined by clusters of protein–protein interactions. NetWeAvers is an R package that provides a novel method for analyzing proteomics data integrated with biological networks. The method includes an algorithm for finding dense clusters of proteins and a permutation algorithm to calculate cluster P-values. Optional steps include summarizing quantified peptide values to single protein values and testing for differential expression, such that the data input can simply be a list of identified and quantified peaks.

Availability and implementation: The NetWeAvers package is written in R, is open source and is freely available on CRAN and from netweavers.erasmusmc.nl under the GPL-v2 license.

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statistical significance for each protein. The resulting $P$-values for individual proteins can be used in the network analysis step, which scores highly connected subgraphs, i.e. dense clusters, with these $P$-values. Because the need to specify many parameters can greatly impact the results, we chose a highly data-driven cluster-finding algorithm that requires only one parameter. Our protein and cluster scoring each require only one additional parameter.

2 DESCRIPTION

NetWeAvers provides a method for the integrated statistical analysis of MS data and biological networks. The input for NetWeAvers is a set of peaks from an MS experiment that has been identified, quantified and normalized. The data can be input as an R/Bioconductor ExpressionSet or a matrix to be converted into an ExpressionSet using customSummarizer. If the data are at the peptide level, then summarization to the protein level is required for use in NetWeAvers (esetSummarizer). This can be done before or after testing for DE (DEtest). The summarization step consists of aggregating all peptide quantities for a given protein using the mean or median so that each protein only has one value per sample.

The test for DE is implemented using the linear modeling framework of the limma package (Smyth, 2004). The output of the test includes $P$-values that may be used in the main algorithm of NetWeAvers (runNetweavers), which maps the proteins to a user-specified network in node–node format and performs the network analysis. The function findDenseClusters uses the Walktrap algorithm for finding highly connected subgraphs as implemented in the R package igraph (Csárdi and Nepusz, 2006) as a part of the network analysis algorithm.

The clusters are scored using a weighted mean or median of log-transformed $P$-values (scoreClusters). The weights are a function of the number of proteins with which a given protein interacts. A permutation test (permTest) may be carried out to determine the statistical significance of the clusters. See Supplementary File 1 for more details on the cluster scoring and the permutation test, as well as Supplementary Figure S1 for a schematic overview of the NetWeAvers procedure.

3 APPLICATION

We applied the R package to MS data from a phosphorylation study of human embryonic stem cells (Van Hoof et al., 2009, see Supplementary File S1 for the experimental design). The R package vignette provided as Supplementary File S2 presents the code for summarizing the data, performing hypothesis testing and running the network analysis using the Reactome human PPIN, version 43 (Croft et al., 2011). NetWeAvers identified clusters of proteins with roles in processes known to be involved in stem cell differentiation. See Supplementary File 1 for these results, results from NetWeAvers applied to a null dataset and an example using data that were summarized and tested in another R package.

4 CONCLUSIONS

NetWeAvers is a unique algorithm designed for quantitative MS data that incorporates key features of the proteins and networks ($P$-values and number of interactors, respectively) being analyzed. It uses only a few parameters and does not arbitrarily filter out non-significant proteins. We applied our method to a publicly available MS dataset and found statistically significant and biologically meaningful networks. The method may also be used with gene expression data. Many databases provide PPINs in node–node format, which makes it easy for users to connect NetWeAvers with their favorite databases. The format of the NetWeAvers output allows for simple connections to tools like Cytoscape (Shannon et al., 2003) to visualize the resulting clusters.

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REFERENCES


