



APPLICATION NOTE

NORMA: The Network Makeup Artist — A Web Tool for Network Annotation Visualization



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Received 5 March 2020; revised 8 July 2020; accepted 20 November 2020

Available online 24 June 2021

Handled by Edwin Wang

KEYWORDS

Network annotation;
Visualization;
Topological analysis;
Community detection;
Functional enrichment
visualization

Abstract The Network Makeup Artist (NORMA) is a web tool for interactive **network annotation visualization** and **topological analysis**, able to handle multiple networks and annotations simultaneously. Pre-calculated annotations (*e.g.*, Gene Ontology, Pathway enrichment, **community detection**, or clustering results) can be uploaded and visualized in a network, either as colored pie-chart nodes or as color-filled areas in a 2D/3D Venn-diagram-like style. In the case where no annotation exists, algorithms for automated community detection are offered. Users can adjust the network views using standard layout algorithms or allow NORMA to slightly modify them for visually better group separation. Once a network view is set, users can interactively select and highlight any group of interest in order to generate publication-ready figures. Briefly, with NORMA, users can encode three types of information simultaneously. These are 1) the network, 2) the communities or annotations of interest, and 3) node categories or expression values. Finally, NORMA offers basic topological analysis and direct topological comparison across any of the selected networks. NORMA service is available at <http://norma.pavlopouloslab.info>, whereas the code is available at <https://github.com/PavlopoulosLab/NORMA>.

Introduction

In the biomedical field, networks are widely used to capture the interactions and associations between bioentities (*e.g.*, proteins, genes, small molecules, metabolites, ligands, diseases,

and/or drugs) in order to unravel how complex systems operate [1]. Despite the great variety of visualization tools to efficiently represent such networks [2,3], visualization still remains a bottleneck as views often become incomprehensible due to a large number of nodes and edges. Therefore, tools for the visualization of communities or areas of interest in a network emerge. Popular state-of-the-art tools include, Cytoscape [4], Cytoscape.js web tool [5], Gephi [6], Pajek [7], Ondex [8], Proviz [9], VisANT [10], Medusa [11], Osprey [12], Tulip [13], Arena3D [14], Arena3D^{web} [15], and Graphia (Kajeka) and BioLayout Express [16]. Nevertheless, most of these tools

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Peer review under responsibility of Beijing Institute of Genomics, Chinese Academy of Sciences / China National Center for Bioinformatics and Genetics Society of China.

<https://doi.org/10.1016/j.gpb.2021.02.005>

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mostly focus on interactivity, layout, and network exploration and lack efficiency in network annotation visualization.

ClueGO [17], for example, a Cytoscape plugin [18], partially solves this problem by using pie-chart nodes to visualize the non-redundant biological terms for large clusters of genes in either a functionally grouped network [19] or a KEGG/BioCarta network [20]. Moreover, Enrichment Map [21] uses similar approaches to visualize gene-set enrichment results. In addition, STRING [22] uses pie-chart nodes to overlay Gene Ontology (GO), KEGG [20], and Reactome [23] pathways on a protein-protein interaction (PPI) network [24]. Osprey mainly builds data-rich graphical representations from GO interaction data and is bound to the BioGRID repository [25]. NAViGaTOR [26], offers among others, a GO annotated matrix view as well as a matrix layout to represent groupings. DyCoNet [27], a Gephi plugin, allows community detection and visualization. CellNetVis [28] is a web tool for the visualization of biological networks placing nodes in the cellular components they belong to. ClusterProfiler [29] is an R package that automates the process of biological-term classification and the enrichment analysis of gene clusters.

Many of these tools try to partially address the annotation visualization problem by using node colors or pie-chart nodes. However, they mainly focus on integrating networks with knowledge from existing repositories. In addition, they require familiarity, are bound to certain databases or are offered in a command-line version.

In this study, we present The Network Makeup Artist (NORMA), a highly interactive web tool that is specially designed to visualize communities and network annotations using alternative representations. NORMA is designed for non-experts and is a tool of general-purpose.

Method

NORMA is a handy web tool for interactive network annotation, visualization, and topological analysis and is able to handle multiple networks and annotations simultaneously. In its current version, the main interface is split into four tabs, namely, upload, network, annotation, and topology.

The upload tab

Users can upload three different file types as input. These are the network file, the annotation file, and the node coloring file.

The network file is an obligatory, 2-column or 3-column (unweighted and weighted, respectively), tab-delimited file containing all connections of an undirected network. For simplicity, self-loops and multiple-edges are eliminated automatically.

The annotation file is an obligatory, 2-column, tab-delimited file, which contains information about the defined groups. The first column contains the group names, whereas the second column contains the node names in that group. This file may contain results from a functional enrichment analysis (*e.g.*, GO or KEGG/Reactome pathways) or a clustering analysis (*e.g.*, MCL output [30,31]).

The node coloring file is an optional, 2-column, tab-delimited file, in which users may directly assign a color to a node in order to encode different types of information. An example could be to highlight gene expression values (*e.g.*, red for a down-regulated gene or green for an up-regulated

gene) or a node category (*e.g.*, yellow for category-1 and blue for category-2). In this file, the first column contains the node names, whereas the second column contains the colors as names or hex codes (*e.g.*, red, green, yellow, blue, orange, #00ff00, #ff0000, #ffff00).

In NORMA, users can upload multiple network and annotation files simultaneously. Each time a network or an annotation file is being uploaded, a name can be given first. Once the network and annotation files have been named and uploaded, users can visualize any network and overlay any of the selected annotations. Users can remove indifferent annotations or networks at any time. Network and annotation file contents are always shown as interactive tables. In these tables, one can search by suffix from the search field.

The network tab

This tab consists of two sub-tabs dedicated to network analysis and visualization. These are the interactive network and the automated community detection.

The interactive network sub-tab offers a dynamic network visualization in its simplest form with the use of the *vizNetwork* library. Nodes are connected with undirected edges, whereas their coordinates are calculated using *vizNetwork*'s default force-directed layout. The network is fully interactive as zooming, dragging, and panning is allowed either by using the mouse or the navigation buttons. Nodes can be selected and dragged anywhere on the plane, whereas the first neighbors of any node can be highlighted upon selection. The network view is automatically updated when a different network from the ones that have been uploaded is selected.

The automated community detection sub-tab offers methods for automatic community detection. This is particularly important in cases where users do not have any external pre-calculated results to provide. The algorithms that are currently offered are: Fast-Greedy [32], which tries to find densely connected communities via directly optimizing a modularity score; Louvain [33], which implements a multi-level modularity optimization algorithm for finding community structures, and is based on the modularity measure and a hierarchical approach; Label-Propagation [34], a fast, nearly linear time algorithm for detecting community structures in a network, which is done by labeling the vertices with unique labels and then updating them by majority voting in the neighborhood of the vertex; Walktrap [35], which tries to find densely connected subgraphs in a graph via random walks, with the idea that short random walks tend to restrict themselves in the same community; and Betweenness [36] where clustering is made by 'breaking' the bridges which connect the densely connected regions of a network.

Once a community detection method has been selected, users can see the results as interactive and searchable tables or as static plots for an at-a-glance view. In order for users to take advantage of NORMA's advanced interactive visualization capabilities, the automatically generated annotations must be first exported and then imported as annotation input files.

The annotation tab

This tab is the NORMA's strongest feature and is used to visualize annotated networks in an easy and user-friendly way.

Annotated are considered the networks which are accompanied by predefined clusters, communities, subgraphs, regions, or neighborhoods. Here, users can select between any of the uploaded networks or annotation files and visualize them in combination (dropdown selection lists). This tab consists of four sub-tabs. These are convex hulls, 3D convex hulls, pie-chart nodes, and Venn diagrams.

Convex hulls

In this sub-tab, the selected network is initially visualized after applying any of the offered layout algorithms. Color-filled convex hulls are then used to highlight communities in a Venn-diagram-like view. Notably, a node might belong to more than one group. Groups are highlighted with visually distinct colors, while transparency is used to efficiently highlight the overlapping regions.

3D convex hulls

Like in 2D convex hulls, in this sub-tab, the selected network is initially visualized in 3D space with the help of *plotly* library, whereas 3D shaded volumes are used to highlight communities in a 3D Venn-diagram-like view.

Pie-chart nodes

Here, the selected network is initially visualized after applying any of the offered layout algorithms, and nodes are then visualized as pie-charts. A pie chart is divided into slices to illustrate the groups a node belongs to. If a node, for example, belongs to four groups, then the pie chart will consist of four equal slices colored with distinct colors. Nodes that do not belong to any group are marked gray.

Node coloring

Often, one might want to assign certain colors to nodes in order to encode certain types of information. In a gene expression network, for example, one might want to highlight the up- and down-regulated genes. Once an expression file has been uploaded, nodes in the “convex hulls” tab will be filled with the color of interest, whereas nodes in the “pie-chart nodes” tab will appear with a colored border. As node coloring is an optional feature, one can enable or disable this functionality at any time (selection box).

To briefly demonstrate this functionality, we queried the STRING database for the human *BCAR3* gene and retrieved its interactions. We used all STRING channels (Text-mining, Experiments, Databases, Co expression, Neighborhood, Gene Fusion, and Co-occurrence) using the medium confidence threshold (0.4) and allowed no more than 10 interactors (first neighbors). After exporting STRING’s KEGG analysis results and importing them into the NORMA application, we highlighted four pathways. These are the protein processing in endoplasmic reticulum (genes involved: *DERL1*, *DERL2*, *NPLOC4*, *NSFLIC*, *SYVN1*, *UFDIL*, *VCP*, and *VIMP*), the regulation of actin cytoskeleton (genes involved: *BAIAP2*, *BCAR1*, *CDC42*, *PAK1*, *PAK2*, *PXN*, *WAS*, and *WASL*), the axon guidance (genes involved: *CDC42*, *PAK1*, *PAK2*, *PARD6A*, and *PARD6B*), and the bacterial invasion of epithelial cells (genes involved: *BCAR1*, *CDC42*, *PXN*, *WAS*, and *WASL*). The network, along with the four selected pathways, is shown in **Figure 1**.

Layouts

Users can choose between several layout algorithms to visualize a network. In its current version, NORMA offers some of the layout algorithms offered by the *igraph* library [37], including Fruchterman-Reingold [38], which places nodes on a plane using the force-directed layout algorithm developed by Fruchterman and Reingold; Random, which places the vertices of the graph on a plane uniformly using random coordinates; Circle, which places vertices on a circle, ordered by their vertex IDs; Kamada-Kawai [39], which places the vertices on a plane by simulating a physical model of springs; Reingold-Tilford [40], a tree-like layout suitable for trees, hierarchies, or graphs without many cycles; LGL, a force-directed layout suitable for larger graphs; Graphopt, a force-directed layout algorithm which scales relatively well to large graphs; Gem [41], which places vertices on a plane using the GEM force-directed layout algorithm; Star, which places vertices of a graph on a plane, according to the simulated annealing Davidson and Harel algorithm; and Grid, which places vertices on a rectangular grid.

For better clarity, NORMA gives the option to slightly modify the selected layout in order to make groups as distinct as possible and avoid unnecessary overlaps which may occur from the original layout. To do this, NORMA starts by introducing one virtual node per group. Then, it connects these virtual nodes with any node which belongs to this group by assigning a heavyweight. Through this way, the virtual nodes will attract the nodes they relate to regardless of the selected layout. After the completion, all of the virtual nodes are then removed. The whole process is schematically shown in **Figure 2**.

Interactivity and visualization

NORMA gives a variety of options for the creation of optimal custom views. Network zoom in/out and panning functionalities are offered while users can interactively drag any node and place it anywhere on the plane. In addition to the visualized networks, groups are shown in an interactive table whose rows are colored accordingly. By selecting one or more groups, one can show the convex hulls as well as the pie-chart nodes of interest. Colored groups (rows) in the table correspond to colored groups in the offered views and *vice versa*. In addition, users have the option to show and hide the labels or only keep the labels of the selected groups. Labels below a certain zoom level are hidden for clarity. Finally, sliders to adjust node and label size as well as the network size are offered.

Venn diagrams

Users are allowed to choose between any pair of nodes and visualize the common groups they belong to, both as a Venn diagram and as a table. As it is not in the scope of NORMA to provide more complex Venn diagrams (*e.g.*, more than three groups), users are encouraged to visit other online applications [42–45].

The topology tab

Inspired by NAP’s [46] and Cytoscape’s topological network analysis features, in this tab, NORMA offers automated topological analysis and direct comparison of topological features

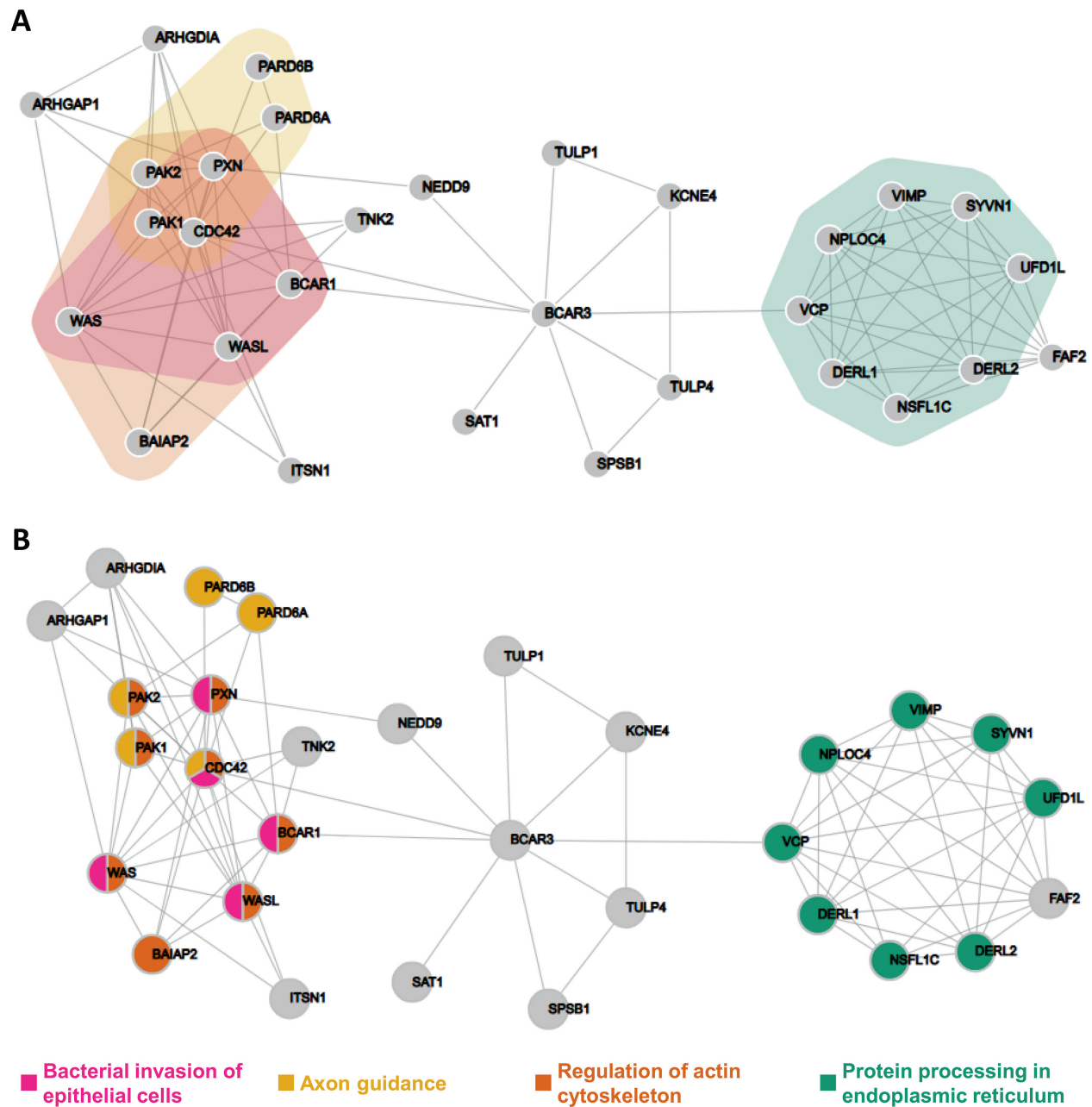


Figure 1 Visualization of *BCAR3* interactors from STRING with four highlighted KEGG pathways

A. Color-filled convex hulls. B. Pie-chart nodes. The networks were generated based on the gene expression data.

between two or more selected networks. The topological measures that are offered by the *igraph* library are as follows: Number of Edges (the number of connections in the network), Number of Nodes (the number of nodes in the network), Density (the ratio of the number of edges to the number of possible edges in a graph), Average Path Length (the average number of steps needed to go from one node to another), Clustering Coefficient (a metric which shows if the network has the tendency to form clusters), Modularity (a function which calculates how modular a given division of a graph into subgraphs is), Average Eccentricity (the distance from a partic-

ular vertex to all other vertices in the graph is first calculated; among those distances, eccentricity is the highest one), Average Number of Neighbors (the total number of neighbors per node divided by the number of nodes), Centralization Betweenness (it is an indicator of the average centrality in a network, and is equal to the number of shortest paths from all vertices to all others that pass through that node; Betweenness centrality quantifies the number of times a node acts as a bridge along the shortest path between two other nodes), and Centralization Degree (the average value of the number of links incident upon a node across all nodes of a network).

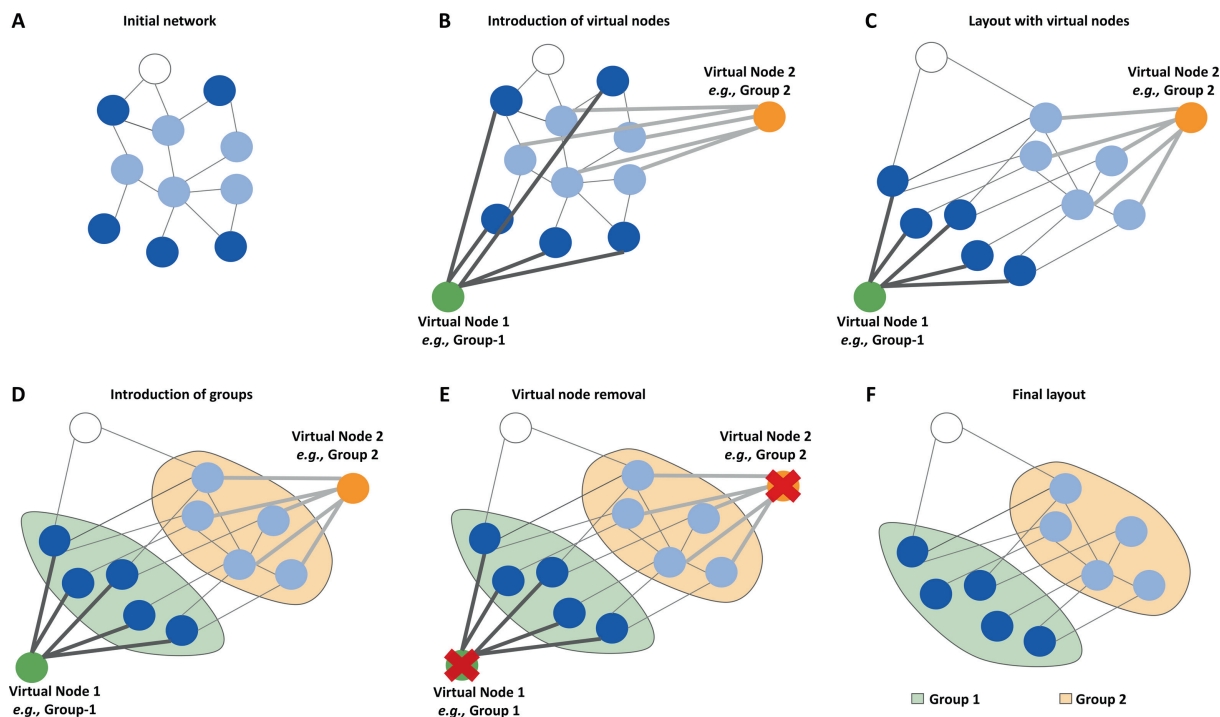


Figure 2 NORMA's layout process

A. NORMA starts with a network using random coordinates. B. Virtual nodes are introduced (one per group) and are connected with heavyweights to the nodes they relate to. C. A selected igraph layout is applied, including the virtual nodes. Due to the heavyweight, virtual nodes are expected to attract the nodes they relate to. D. Once the selected layout algorithm has converged, the color-filled convex hulls are introduced. E. Virtual nodes are removed. F. The final network view.

The topology tab is divided into two sub-tabs: the summaries and the comparative plots. The “summaries” sub-tab shows the aforementioned topological measures in a numerical form as a table. Users can select one or more topological measures of interest and expand the table accordingly. Notably, this can be done for one network at a time upon selection (dropdown selection list). In the “comparative plots” sub-tab, users can directly compare the topological features of two or more networks simultaneously. In contrast to the previous tab, users are allowed to select one topological feature at a time (radio buttons) but as many networks as they like (checkboxes). Once two or more networks and one topological feature have been selected, direct comparisons can be made by the generated bar charts. A slider to adjust the chart height is offered.

Implementation

NORMA's backend is implemented in R, and its frontend in Shiny. Network analysis features are based on the igraph library. Network interactivity and visualization are implemented in d3.js. The 3D network and convex hull visualization are offered by plotly.

A general overview

In order to summarize NORMA's functionality, we briefly show its features in **Figure 3**. The network has been generated by querying STRING for human *TP53* gene (default settings). Figure 3A shows the unweighted network in its simplest form.

Figure 3B shows a static version of the same network clustered with NORMA's Louvain clustering algorithm. Figure 3C shows the network and its groups (manually and randomly assigned) both as color-filled convex hulls and as pie-chart nodes. Groups are also shown as a table (legend), and colors between the network views and the table are consistent. In both views, node coloring encodes additional information (full color in the case of convex hulls or border color in the case of pie-chart nodes). Figure 3D shows a Venn diagram to highlight the common groups between two selected nodes. Figure 3E shows the topological network analysis of the *TP53* network and a direct comparison (e.g., the number of edges) with the *BCARC3* network described in Figure 1.

Results

In order to demonstrate NORMA's capabilities in a real case study, we used the experimental data described in [47]. Null *tau* mutants in *Drosophila* were used to demonstrate broad changes in their brain proteome with mass-spectrometry. To briefly describe the protocol used in this study, *tau*^{KO} mutant flies [48] and Cantonised-w1118 control flies were cultured on standard wheat-flour-sugar food supplemented with soy flour and CaCl₂, at 25 °C in 50%–70% relative humidity in a 12 h light/dark cycle. Three to four biological and two technical replicas from each genotype were used with each biological replica counting 10 brains. The samples were digested following the filter-aided sample preparation (FASP) method using spin filter devices with a 10 kDa cutoff (Sartorius, VN01H02) [49]. The peptide products were detected by an

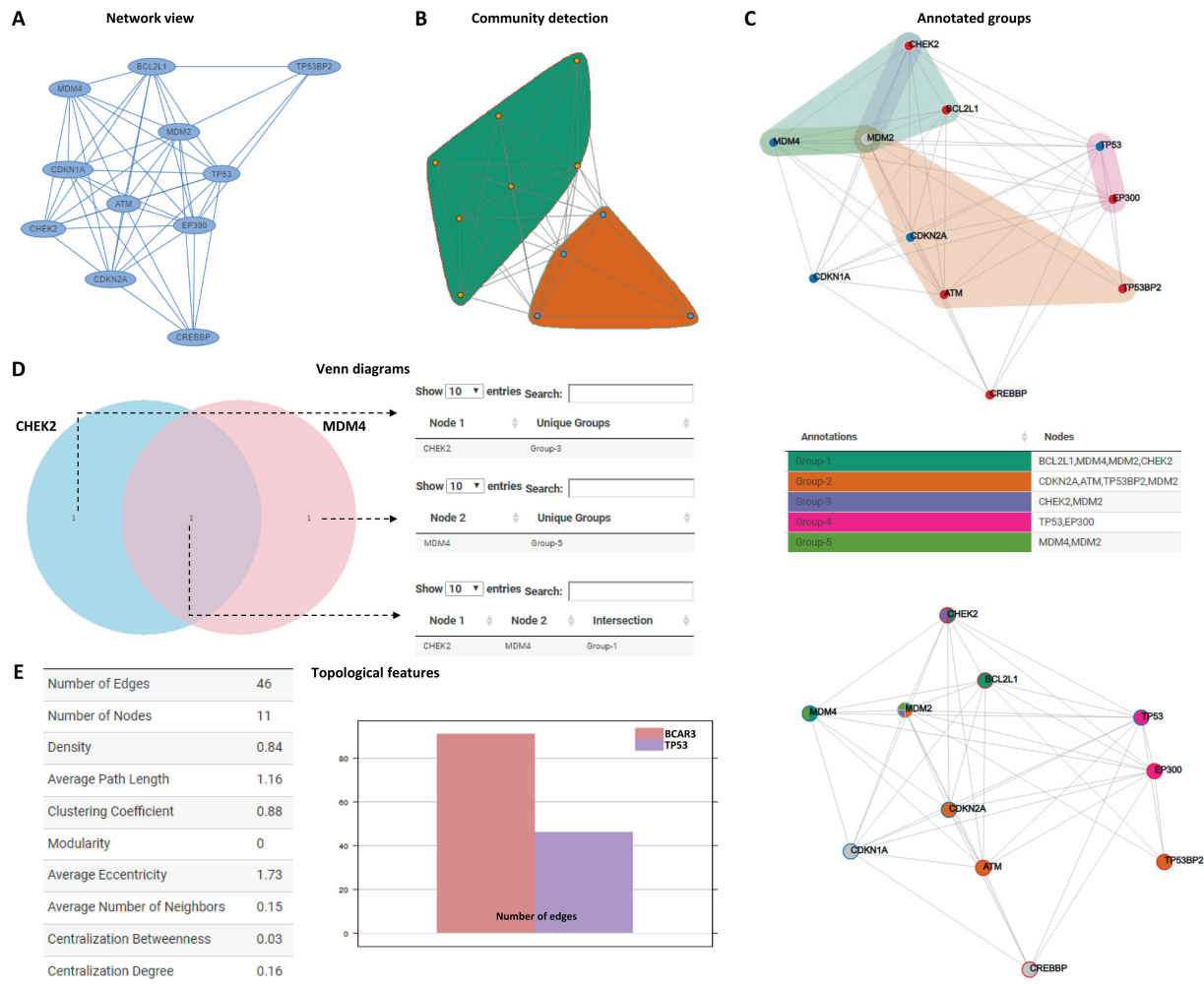


Figure 3 NORMA's overview

A. The *TP53* network. **B.** Community detection using the Louvain algorithm. **C.** Upper: convex hulls with colored nodes. Lower: pie-chart nodes with border colors. Middle: groups visualized in an interactive table (group selection/isolation is allowed). **D.** Venn diagram showing common groups between any pair of selected nodes. **E.** Left: basic topological analysis of the *TP53* network. Right: bar chart to directly compare one topological feature between two networks. The networks were generated based on the gene expression data.

LTX Orbitrap XL mass spectrometer (ThermoFisher Scientific, Waltham, MA) as described elsewhere [50]. The mass spectral files (raw files) were processed using MaxQuant software (version 1.5.3.30) [51] against the complete UniProt proteome of *Drosophila melanogaster* (downloaded April 1, 2016; 42,456 entries) and a common contaminant database by the Andromeda search engine. For the calculation of the protein abundances, label-free quantification (LFQ) was performed with both “second peptides” and “match between run” options enabled. Statistical analysis was performed using Perseus (version 1.5.3.2). The label-free quantified proteins were subjected to statistical analysis with the Student's *t*-test (permutation-based *P* value with 0.05 cutoff).

Starting from the identified 503 differentially expressed proteins, we used STRING to extract the connections among 358 of them and performed STRING's KEGG analysis to highlight several important pathways (Figure 4). Up- and down-regulated proteins are marked in green and red accordingly. The most affected pathways upon *Drosophila tau* (*dTau*) loss in the central brain are shown as color-filled convex hulls and involve molecular processes reported altered in

Tauopathies. Tau, for example, is known to bind to ribosomes in the brain and thus impair their function by reducing protein synthesis [52], an effect enhanced in Tauopathy brains [53]. In agreement, loss of *dTau*, resulted in elevated translation and protein synthesis as reflected by the increased expression of translation machinery components, including initiation factors, proteins that control gene expression or silencing, and ribosomal proteins. Spliceosome disruption and altered pre-mRNA processing are also emerging as potential contributors to *Tau*-mediated neurodegeneration [54]. On the other hand, transgenic *Tau* elevation by expression of human *Tau* in the fly central nervous system, triggered the reduction of multiple spliceosomal proteins [55]. In accordance with these results, several RNA-binding proteins and spliceosome components are more abundant in the mutant. Congruently with translation, proteins mediating proteasomal catabolism are equally elevated. Interestingly, proteasome activation can be viewed as an adaptive response to support elevated protein synthesis by increasing the size of the intracellular amino acid pool [56]. Finally, proteins implicated in energy-metabolism (oxidoreductases) and responsiveness to oxidative stress,

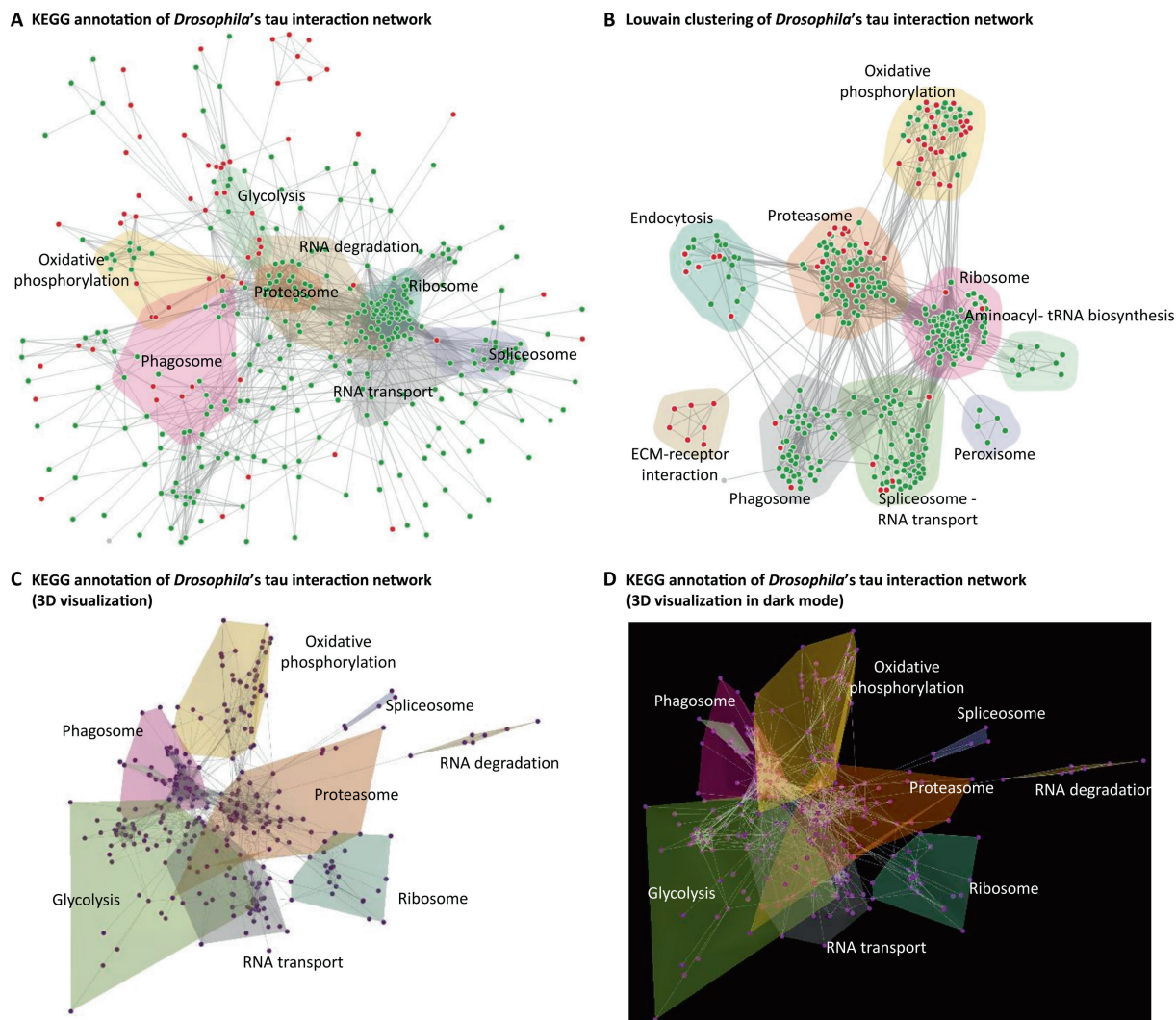


Figure 4 *Drosophila*'s tau interaction network

A. Eight major KEGG pathways highlighted. **B.** Louvain clustering and functional annotation with DAVID application. **C.** The previous result was visualized by NORMA in 3D space. **D.** The latter in dark mode. In (A) and (B), up- and down-regulated proteins are marked in green and red, respectively.

appear as differentially regulated in the mutants, with about half of them attenuated by its loss. This is consistent with the reported accumulation of oxidative damage markers in Tauopathy patients [57] and the effect of oxidative stress in mediating *tau*-induced neurotoxicity in flies [58] (Figure 4A).

To partially regenerate the annotation in Figure 4A, we used the NORMA's Louvain community detection algorithm to cluster the network based on its topology only and the DAVID [59] application to perform pathway and GO enrichment analyses. In Figure 4B, results revealed nine distinct communities, which are highlighted. Within these communities, 4/22 proteins are involved in endocytosis, 21/75 in the proteasome, 3/5 in the peroxisome, 43/85 in the ribosome, 15/58 in the spliceosome, 15/49 in oxidative phosphorylation, 4/12 in the extracellular matrix (ECM) receptor, 8/47 in the phagosome, and 7/8 in aminoacyl-tRNA biosynthesis. Figure 4C and D show the same information as in Figure 4B in 3D space in light and dark modes, respectively.

Discussion

NORMA is a web tool for network annotation visualization. It is able to handle multiple annotations and networks simultaneously and is made to aid researchers in producing high-quality publication-ready figures as well as to explore novel hypotheses. In its current version, NORMA is not bound to a certain database or repository, and in the future, it will come with alternative visualization options (e.g., circo-like diagrams with edge bundling support). At the moment, NORMA's online version supports networks of up to 10,000 nodes, a limitation that can be removed in its offline version. We believe that due to its interactivity and ease of use, NORMA is a handy tool for selective annotation visualization designed for non-experts without prior familiarity with command-line tools or scripting. To highlight NORMA's advantages, we compared it with other applications in Table S1 and used it to show how it can complement alterna-

tive software like the ClueGO (Figure S1). The dataset used was a *Gallus gallus* BioGrid [25] PPI analyzed by DAVID functional annotation tool.

Code availability

The code is available at <https://github.com/PavlopoulosLab/NORMA>.

Data availability

NORMA service is available at <http://norma.pavlopouloslab.info>.

CRedit author statement

Mikaela Koutrouli: Methodology, Software, Validation, Visualization, Writing - original draft, Writing - review & editing. **Evangelos Karatzas:** Software, Validation, Writing - review & editing. **Katerina Papanikolopoulou:** Formal analysis, Validation, Writing - review & editing. **Georgios A. Pavlopoulos:** Conceptualization, Supervision, Methodology, Writing - original draft, Writing - review & editing. All authors have read and approved the final manuscript.

Competing interests

Authors declare that there is no conflict of interest.

Acknowledgments

Georgios A. Pavlopoulos and Mikaela Koutrouli were supported by the Operational Program Competitiveness, Entrepreneurship, and Innovation, NSRF 2014-2020 (Grant No. MIS 5002562, co-financed by Greece and the European Union (European Regional Development Fund)). Georgios A. Pavlopoulos was also supported by the Hellenic Foundation for Research and Innovation (HFRI) under the “First Call for HFRI Research Projects to support Faculty members and Researchers and the procurement of high-cost research equipment grant” (Grant No. 1855-BOLOGNA). Evangelos Karatzas was supported by the Action Strengthening Human Resources, Education and Lifelong Learning, 2014–2020, co-funded by the European Social Fund (ESF) and the Greek State (Grant No. MIS 5000432). Katerina Papanikolopoulou was supported by a grant from the Stavros Niarchos Foundation to the Biomedical Sciences Research Center “Alexander Fleming”, as part of the initiative of the Foundation to support the Greek research center ecosystem. We thank Dr. Yorgos Sofianatos for his valuable feedback and Dr. Alexandros Dimopoulos for the server setup.

Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.gpb.2021.02.005>.

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