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Applications Note

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DyNet: visualization and analysis of dynamic molecular interaction networks

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Abstract

Summary: The ability to experimentally determine molecular interactions on an almost proteome-wide scale under different conditions is enabling researchers to move from static to dynamic network analysis, uncovering new insights into how interaction networks are physically rewired in response to different stimuli and in disease. Dynamic interaction data presents a special challenge in network biology. Here, we present DyNet, a Cytoscape application that provides a range of functionalities for the visualization, real-time synchronization and analysis of large multi-state dynamic molecular interaction networks enabling users to quickly identify and analyze the most 'rewired' nodes across many network states.

Availability and Implementation: DyNet is available at the Cytoscape (3.2+) App Store (http://apps.cytoscape.org/apps/dynet).

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Supplementary Information: Supplementary data are available at *Bioinformatics* online.

1 Introduction

Network biology is a rapidly developing area of research, which recognizes that cellular processes are controlled and coordinated at multiple different levels by tightly regulated molecular networks. This cellular 'interactome' is a highly dynamic entity, dependent on an array of temporal, spatial, cellular and environmental signals (Barrios-Rodiles *et al.*, 2005; Przytycka *et al.*, 2010). Understanding how molecular interaction networks change in their composition and connectivity in response to these cellular cues is crucial to understanding complex emergent phenotypes in health and disease (Barabási *et al.*, 2011; Bowler *et al.*, 2015). Sahni *et al.* (2015), for example have shown that two thirds of disease-associated alleles perturb protein–protein interactions (PPIs). Until recently, most studies investigating the dynamics of PPI networks inferred dynamic network states through the integration of static maps of the interactome with state-specific information, such as gene expression or genetic interaction data (Fischer *et al.*, 2015; Will and Helms, 2015). Fortunately, advances in Mass Spectrometry are now enabling high-throughput studies that directly investigate PPI network dynamics (Ideker and Krogan, 2012). Recently, for example researchers have mapped the Hippo signaling pathway PPI network and revealed how changes in protein phosphorylation result in a significant rewiring of the interactions between members of this pathway (Couzens *et al.*, 2013). Dynamic interaction data presents a special challenge in network visualization and current software tools are limited in their ability to visualize and analyze this type of data. The *DynNetwork* Cytoscape application (http://apps.cytoscape.org/apps/dynnetwork), for example uses animation to visualize changes in networks over time/conditions. While fun, this approach is of limited practical utility especially for larger or highly dynamic networks and the user can quickly lose track of the nodes/edges of interest. This approach also lacks statistical measures to support the detection and quantification of network rewiring. The *kDDN* approach (Tian *et al.*, 2015) is more advanced statistically but lacks integration with advanced network visualization tools and is limited to the comparative analysis of only two network states. Here, we present

DyNet, a Cytoscape application that provides a range of functionalities for the visualization and analysis of large multi-state dynamic molecular interaction networks and the identification of the most rewired nodes.

2 Approach

DyNet represents a dynamic network as a set of state graphs (one for each condition, time-point, etc.) and provides a new Cytoscape feature to import multiple graph files in an automated manner. Once imported, the initial network layout (which can be chosen from any Cytoscape layout algorithm), and any subsequent editing, of each state graph is managed and synchronized in real-time via a central reference network (constructed from the union of all states). This means that corresponding nodes and edges will always be located in the same relative positions in each graph view, even during user editing (e.g. dragging, zooming, moving a node), thus making it easier for a user to visually compare graph states and keep track of the changes in nodes/edges of interest across large, many-state dynamic networks. The central reference network also fulfils a second role as a canvas for the visualization of comparative node/edge statistics (see Fig. 1). DyNet provides two principal modes of analysis; one for pairwise ($N = 2$) comparisons and the other for comparisons of multiple ($N > 2$) network states. Each mode supports the visualization of node/edge changes, based on their presence/absence or the value of a selected numeric attribute (e.g. node abundance, edge weight), across networks (Fig. 1). When comparing numeric attributes between two networks (*pairwise mode*), DyNet calculates the log_2 fold-change of the attribute value. When comparing more than two networks a different approach must be taken to produce a summary statistic of node/edge rewiring. Here, we calculate the normalized variance for the numeric attribute across all networks. In either case, the resulting score is used to highlight the most variable nodes and edges on the central reference network, using a color gradient, and added as an attribute in Cytoscape's table view. Additionally, we also define a rewiring metric, the D_n-score, to support the identification of the most rewired nodes in a dynamic network or, more specifically, the most dynamic *neighborhoods*, i.e. the induced sub-graph consisting of all nodes adjacent to a given node. Node rewiring can be defined both in terms of changes in the identity of interacting neighbors (e.g. protein A interacts with different proteins under different conditions) and/or in terms of changes in the edge weights for a node's connections (e.g. quantitative changes in the affinity of protein A and its interacting partners over different conditions, detected, for example, using SILAC). A dynamic network may be modeled as a weighted node adjacency matrix that is then extended by a third dimension, S, to describe the state-space, i.e. $M(P, Q, S)$. In this model, the dynamic rewiring score, D_n-score, of a node may be calculated based on the variance of its corresponding row, $M(p, Q, s)$, over the various network states, S, relative to the mean (centroid, c), where the base dissimilarity measure (d) is the *Euclidean* distance (see Equation 1 and Supplementary Fig. S1 for further details, including a simple worked example). The D_n-score is defined as:

$$D_n(NodeX) = \frac{\sum_{i=1}^{S} d(x_i, c)}{S - 1} \quad (1)$$

where the vector x_i represents the neighborhood $M(p, Q, s_i)$ after standardization. The D_n-score favors high degree nodes or *hubs* (which often prove more interesting biologically) and a degree corrected version is also provided. High scoring rewired nodes are highlighted on the central reference network and on each state graph, via node color, and added as a new node attribute in Cytoscape's table view. DyNet also offers additional ancillary features such as the *Node Analyzer*, which enables a chosen node neighborhood be viewed in isolation both in the central reference network and across all states. This is especially useful for untangling the dynamics of large, densely connected, many-state networks. DyNet also has an advanced filtering option and an alternative method to visualize dynamic edge behaviour in the form of an 'edge-by-state' heatmap and associated dendrogram that can be viewed in parallel with the main graph-based visualizations. DyNet is implemented as a cross-platform Cytoscape app (3.2 and above) (http://apps.cytoscape.org/apps/dynet).

To demonstrate DyNet's performance we present two case studies. Couzens *et al.* (2013) recently mapped the Hippo signalling pathway PPI network in the presence and absence of stimulation by okadaic acid (OA). DyNet was used to visualize and analyze these two states of the Hippo pathway network and to identify the most rewired nodes (see Fig. 1 and Supplementary Information). DyNet highlighted Lats2 (large tumor suppressor kinase2) as one of the

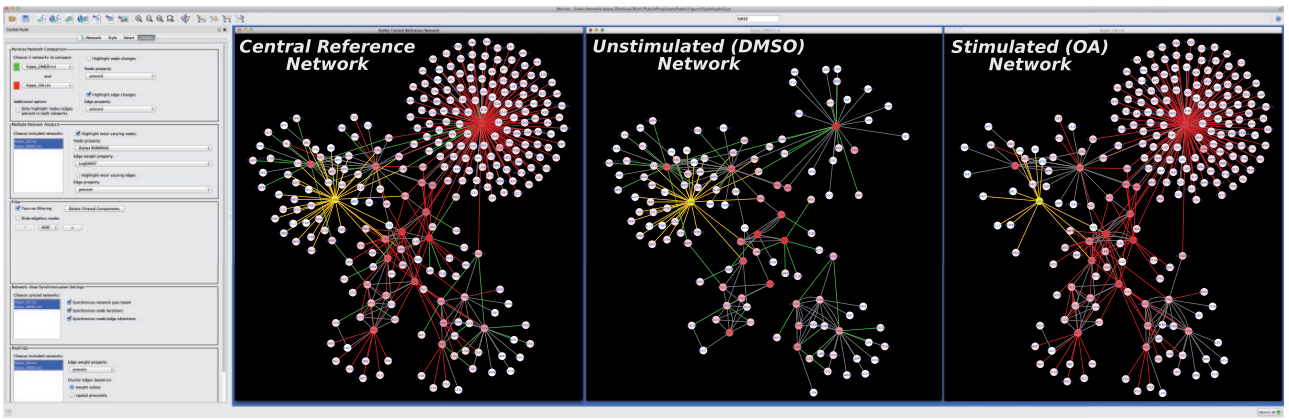

Fig. 1. A DyNet visualization of the Hippo signalling pathway protein–protein interaction network in the presence and absence of okadaic (OA), an inhibitor of serine and threonine phosphatases (Couzens *et al.*, 2013). The network window on the left shows the central reference *union* network—red edges are present only upon stimulation (OA); green edges are present only in the unstimulated network (DMSO); grey edges are present in both. DyNet highlights nodes which are most rewired (more red = higher variation) via the *D_n-score*, see text. The Lats2 protein, for example, shows significant rewiring of its adjacent neighbours following OA treatment (yellow sub-network)

nodes that was notably rewired in the presence of OA. In the absence of OA, Lats2 had 32 interacting partners. The majority (24) were lost following stimulation with OA, while 6 new interactions were gained. The authors of the original study suggested this rewiring represented a shift from repressive interactions to those that promote LATS activity, such as MST1 and MST2. Interestingly, DyNet also identified SAV1 (scaffolding protein salvador homolog 1) as being notably rewired, which has also been shown to associate with MST1 and MST2 to activate the Hippo signaling pathway (Harvey *et al.*, 2013). To showcase DyNet's ability to handle larger, multi-state networks we have also inferred a large-scale dynamic Epidermal Growth Factor Receptor (EGFR) pathway PPI network across 47 different tissues (where each state network has 453 nodes and 588 edges on average), see Supplementary Information. DyNet took ~10 s to import all 47 tissue-specific networks, via its multiple file import feature, and ~20 s to build and lay out the central reference network, all state networks and to identify the most rewired nodes (16Gb RAM; Core i5). DyNet identified several known regulators of EGFR signaling (EGFR, RAF1, GRB2) as being the most rewired across tissues (Supplementary Information and Fig. S2). We have also tested DyNet with a simulated dataset containing 100 network states, with on average 1300 nodes and 2,200 edges per state, which represents the approximate upper analysis limit.

3 Conclusion

DyNet introduces several tools and metrics for visualizing and analyzing node and edge rewiring within large-scale, many-state, dynamic molecular interaction networks. We believe DyNet will be invaluable to researchers as they continue to generate large-scale contextual interaction data and delve into the rewiring of the cell's dynamic interactome.

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Conflict of Interest: none declared.

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