FLUXVIZ — CYTOSCAPE PLUG-IN FOR VISUALIZATION OF FLUX DISTRIBUTIONS IN NETWORKS

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Motivation: Methods like FBA and kinetic modeling are widely used to calculate fluxes in metabolic networks. For the analysis and understanding of simulation results and experimentally measured fluxes visualization software within the network context is indispensable.
Results: We present FluxViz, an open-source Cytoscape plug-in for the visualization of flux distributions in molecular interaction networks. FluxViz supports (i) import of networks in a variety of formats (SBML, GML, XGMML, SIF, BioPAX, PSI-MI) (ii) import of flux distributions as CSV, Cytoscape attributes or VAL files (iii) limitation of views to flux carrying reactions (flux subnetwork) or network attributes like localization (iv) export of generated views (SVG, EPS, PDF, BMP, PNG). Though FluxViz was primarily developed as tool for the visualization of fluxes in metabolic networks and the analysis of simulation results from FASIMU, a flexible software for batch flux-balance computation in large metabolic networks, it is not limited to biochemical reaction networks and FBA but can be applied to the visualization of arbitrary fluxes in arbitrary graphs.
Availability: The platform-independent program is an open-source project, freely available at http://sourceforge.net/projects/fluxvizplugin/ under GNU public license, including manual, tutorial and examples.

Keywords: Cytoscape; fluxes; visualization; systems biology; metabolic network; FBA.

1. Introduction

Software to visually explore biological networks plays a key role in the development of integrative biology, systems biology and bioinformatics. Many tools for visualization of biological networks are available including widely-used examples such as Cytoscape, VisANT, Pathway Studio and Patika [1, 18]. These tools serve, besides their main task to support visual exploration of network structure, also as platform for integration and visualization of data from experiments, simulations and bioinformatic analysis. One such data type is flux information in biological networks like metabolic fluxes in metabolic networks or information fluxes in signal transduction networks. The flux distributions can be based on experimental methods like pulse labeling or flux sensors [14] or result from simulations like FBA [8, 12] or kinetic modeling [13]. Visualization tools for flux information in the network context are essential and should implement

(i) *import* of networks and flux distributions in a variety of formats.
(ii) *batch* analysis of multiple flux distributions in a consistent network layout with simple switch between the different flux distributions.
(iii) generation of *subnetwork views* based on varying network attributes like flux values or localization.
(iv) a *flexible mapping system* between flux values and visual network properties like edge weight or color.
(v) support for the *integration and visualization of additional information* like localization or gene expression data.
(vi) *export* of generated network views in a variety of formats.

Based on the stated requirements the available tools all have mayor limitations (Table 1). CellNetAnalyzer [10] has only minor visualization capabilities and no support for batch analysis, subnetwork views, flexible mappings, visualization of additional data and export of network views. Furthermore it is based on the commercial software package Matlab. FBA-SimVis [3], a VANTED [7] plug-in for FBA simulations with integrated visualization, lacks batch analysis and export, subnetwork views and flexible mappings. FaBina [11] implements only basal subnetwork generation features like flux within single user-defined pathways or compartments. Consistent layouts between different flux distributions, flexible mapping functions and advanced subnetworks are missing. The VisANT flux visualization tool (FVT) [17] and YANAsquare [15] lack among other things crucial import and export features. Specialized tools for the visualization of biological networks like Cytoscape [9] or VisANT [6] have advanced mapping and import systems but do not implement visualization of flux distributions.

2. Results

We present FluxViz, an open-source Cytoscape plug-in for the visualization of flux distributions in networks. Cytoscape is an open-source bioinformatics software platform for visualizing molecular interaction networks and integrating these interactions with gene expression profiles and other state data. FluxViz extends the basic Cytoscape capabilities with flux visualization features (Table 1). FluxViz was primarily developed as a tool for the visualization of fluxes in metabolic networks and as frontend for FASIMU [5], a flexible software for flux-balance computation series in large metabolic networks, and uses the generated output files as input for visualization. FluxViz is not limited to biochemical reaction networks and FBA but can be applied for the visualization of fluxes in arbitrary graphs. The general workflow using FluxViz is depicted in Fig. 1. FluxViz visualization is solely based on network structure and flux data and therefor independent of the underlying simulation platform or experimental setup which generated the flux data. After import a standard NetworkView is generated which can be adapted and modified by application of layouts (automatic layout algorithms or manual), by changing the mapping properties between network attributes (data associated with network nodes and edges)

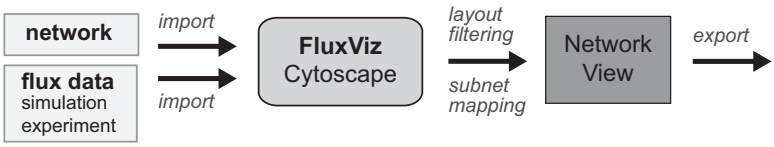


Fig. 1. FluxViz workflow. Network information and flux data can be imported in a variety of formats. A standard NetworkView is generated for the flux distributions in the network context. The NetworkView can be modified by application of layout algorithms, filtering network data, generation of subnetworks based on arbitrary node attributes or flux information and definition of additional visual mappings. The used (flux edge attribute) mapping function can be adapted and additional information like gene expression data can be mapped on visual network attributes. The resulting NetworkViews can be exported in a variety of formats.

and visual network attributes (visual representation of nodes and edges like color or size) and by selecting subnetworks based on filters. The resulting NetworkViews can be interactively analyzed in Cytoscape or be exported as images.

2.1. *FluxViz features*

Import

Flux distributions can be imported from CSV, Cytoscape attribute or FASIMU val files. Networks can be imported as SBML, GML, XGMML, SIF, BioPAX, PSI-MI or can be manually generated in Cytoscape. Existing modeling tools like Matlab or FASIMU can be easily adapted to generate the CSV flux formats.

Layout

Multiple automatic layout algorithms are available in Cytoscape and can be applied in FluxViz. Manual layouts are also supported. The layout information is a global property for all flux distributions and the network, which enables a simple comparison between flux distributions (see Fig. 2 for an example).

Batch

FluxViz supports the work with multiple flux distributions in one session. Furthermore batch import of files and batch export of NetworkViews has been implemented. In response to selection of a flux distribution the corresponding NetworkView is generated on the fly.

Subnetworks and filtering

FluxViz supports the filtering of NetworkViews based on flux values or node attributes and the generation of subnetworks based on the filter selections. Hereby the NetworkView can for example be constraint to the flux containing subgraph

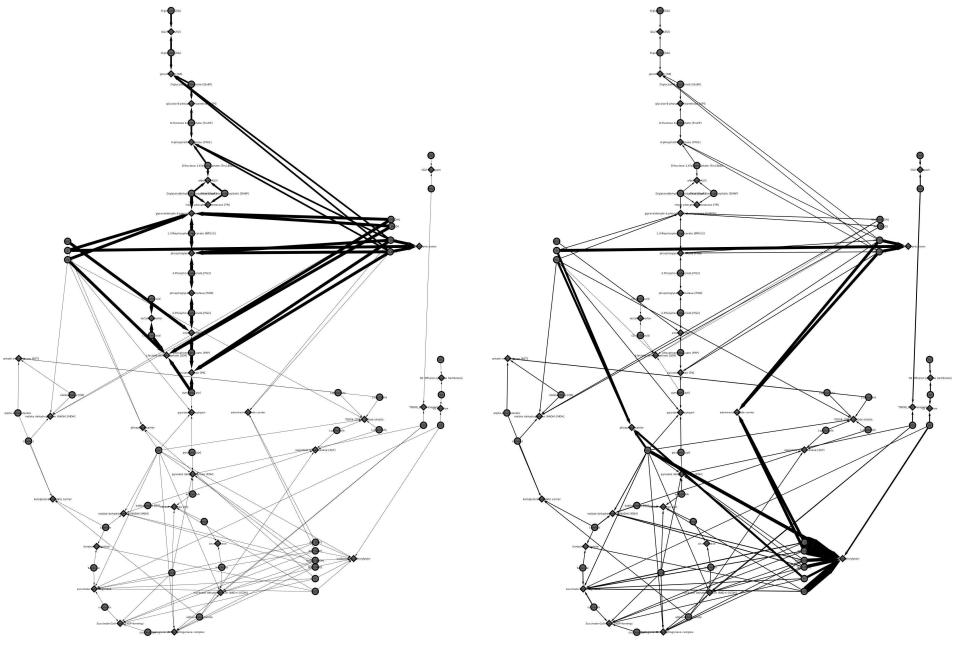
Table 1. FluxViz features in comparison with alternative visualization tools. [+] feature supported, [−] feature not supported, [±] feature partially supported or only basal implementation.

Feature	CellNet–Analyzer	FBA–SimVis	fa–BINA	VisANT FVT	YANA square	Cyto–scape	Flux–Viz	FluxViz Details
Network import	−	+	+	−	+	+	+	many formats (SBML, GML, XGMML, SIF, BioPAX, PSI−MI)
Flux data import	+	+	+	−	−	−	+	CSV format, FASIMU val files, Cytoscape attributes
Export flux distribution views	−	+	+	+	−	+	+	many formats (PDF, SVG, EPS, JPEG, PNG, BMP)
Batch export	−	−	+	−	−	−	+	batch export of selected flux distributions in many formats
Filtering and subnetwork views	−	−	±	−	±	−	+	flux containing network attribute based subnetworks (like compartment or pathway) flux containing attribute networks
Flux mapping on visual attributes	−	+	+	+	+	−	+	all node and edge attributes can be utilized edge size, direction and tooltip used for default visualization
Flexible mapping functionality	−	−	±	+	−	+	+	node and edge attributes for visualization of additional data like localization as node color or gene expression as node size.
Adaptable mapping functions	−	+	−	+	−	+	+	global (all distributions) vs. local (single distribution) settings linear and stepwise linear mappings based on setpoints
Batch support for flux distributions	−	−	±	−	−	−	+	batch import and export cycling through flux distributions on the fly generation of views with consistent layout
Functional enrichment	−	±	±	+	±	+	+	many Cytoscape plug−ins available, large community simple enhancement through plug−in architecture

or can be limited based localization information to generate compartment subnetworks. The flux distributions can be analyzed in small subgraphs separately. This feature is especially important for the analysis of large-scale networks (Fig. 3) which are difficult to analyze as complete graph.

Flexible mapping

FluxViz supports flexible mappings of flux information to the visual node and edge attributes of the NetworkView. In the standard mapping the edge size, direction and the tooltip are used to represent the flux through the corresponding edge. All node and edge attributes can be used to represent additional network information like localization of nodes or gene expression for proteins. In this process the mapping functions between network attributes and visual network attributes can be adapted. After selection of flux distributions the visual edge and node attributes of



(a) (b)

Fig. 2. Example of FluxViz visualization of flux distributions. (a) Glucose utilization under aerob conditions. ATP is only generated in glycolysis, the resulting pyruvate is converted to lactate and exported. (b) Glucose utilization under anaerob conditions. ATP is mainly generated by oxidative phosphorylation, no lactate is exported, much less glucose is needed for the same ATP production. Flux distributions are FASIMU FBA results in human hepatocyte network consisting of glycolysis, gluconeogenesis, pentose phosphate pathway and citrate cycle (supplementary information). Flux minimization [4] was used as objective function with ATP production as target flux under varying oxygen availability. Manually generated consistent layout for comparison, linear (linear flux edge weight) global mapping function.

the NetworkView are changed according to the selected distribution (Fig. 2, Fig. 3).

Export

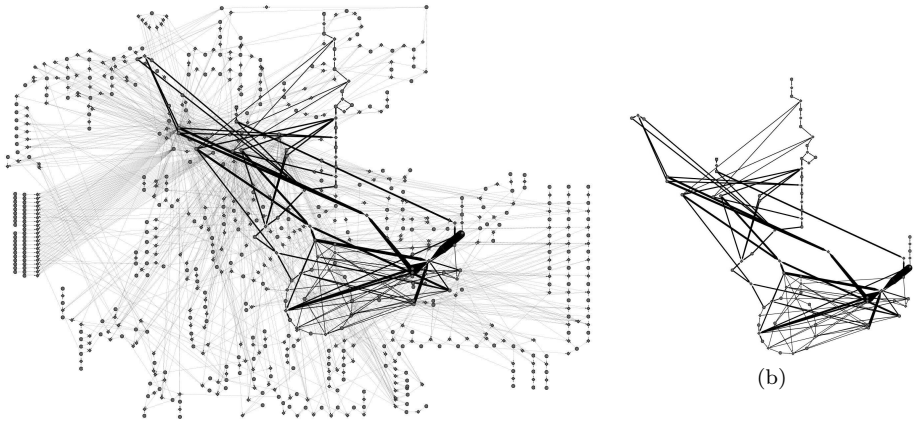
The generated NetworkViews can be exported in a variety of formats (PDF, SVG, EPS, JPEG, PNG, BMP). Batch export is supported.

Integration into well established platform

FluxViz is implemented as an extension of the well established visualization and analysis platform Cytoscape [9]. Therefore many existing features and plug-ins, like network loaders, automatic layout algorithms, advanced filtering mechanisms or network analysis tools, are available and can be utilized in combination with FluxViz.

2.2. *Application*

FluxViz has been applied for kinetic models simulated in Matlab and FBA simulations in FASIMU (Fig. 2, Fig. 3). Furthermore, FluxViz was used for the reconstruction and analysis of a human hepatocyte core network (Fig. 3(a)) and for the visualization of FBA simulations in the reconstruction of the complete human hepatocyte network [2].



(a) (b)

Fig. 3. Filtering and subnetwork features. For the analysis of flux distributions in medium to large-scale networks limitation of network views based on selected attributes is crucial. (a) Flux distribution in the full network (b) flux subnetwork with much lower complexity. Flux distribution is FASIMU FBA result in a reconstructed human hepatocyte network (supplementary information). Flux Minimization [4] was used as objective function in the analysis of glucose formation from oxalacetate. Manually generated Layout, linear (linear flux edge weight) mapping function. Subnetwork is flux subnetwork and consists of edges and nodes with non-zero flux. FluxViz subnetworks can be generated based on arbitrary network attributes like flux values or node localization.

3. Summary

FluxViz provides the necessary tools for the visualization of flux distributions in networks. FluxViz is a visualization tool solely based on network structure and flux data and is therefore independent of used simulation platforms or experimental setups which generate the flux distributions. As a result FluxViz can easily be integrated in existing simulation workflows. Due to features like attribute- and flux-subnetworks and consistent layouts for the loaded networks even genome-scale networks can be analyzed and simulations under varying conditions be compared. Especially for large networks a visual inspection of flux simulation results is an important step in the validation of the network capabilities and the iterative improvement of network reconstructions and models. Due to comfortable mapping capabilities of additional data, flux simulations can be easily visualized in the context of additional information like gene expression values in combination with resulting fluxes in a network [16].

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