

Manual

FluxMap

a VANTED Add-on for the visual exploration
of flux distributions in biological networks

<http://www.vanted.org/fluxmap>

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1 Introduction

FLUXMAP is an easy to use tool for the advanced visualization of simulated or measured flux data in biological networks. Flux data import is achieved via a structured template basing on intuitive reaction equations. Flux data is mapped onto any network and visualized using edge thickness. Various visualization options and interaction possibilities enable comparison and visual analysis of complex experimental setups in an interactive way. The main features of FLUXMAP are:

- visualization of flux data as edge thickness (either simulated or measured)
- enables to model (beside mass fluxes) also N-, C-, and any other fluxes using substance weights
- data input via a structured template based on biochemical equations
- quality of flux measurements (e.g. confidence) can be included and represented by edge color graduation
- provides various visualization parameters such as global edge thickness, arrowhead/-tail ratio, and style of reaction nodes
- compare and explore flux distributions by switching through conditions (species, treatment, genotype etc.) and time (developmental stages etc.)

Two example flux map can be seen in [Fig. 1.1](#)

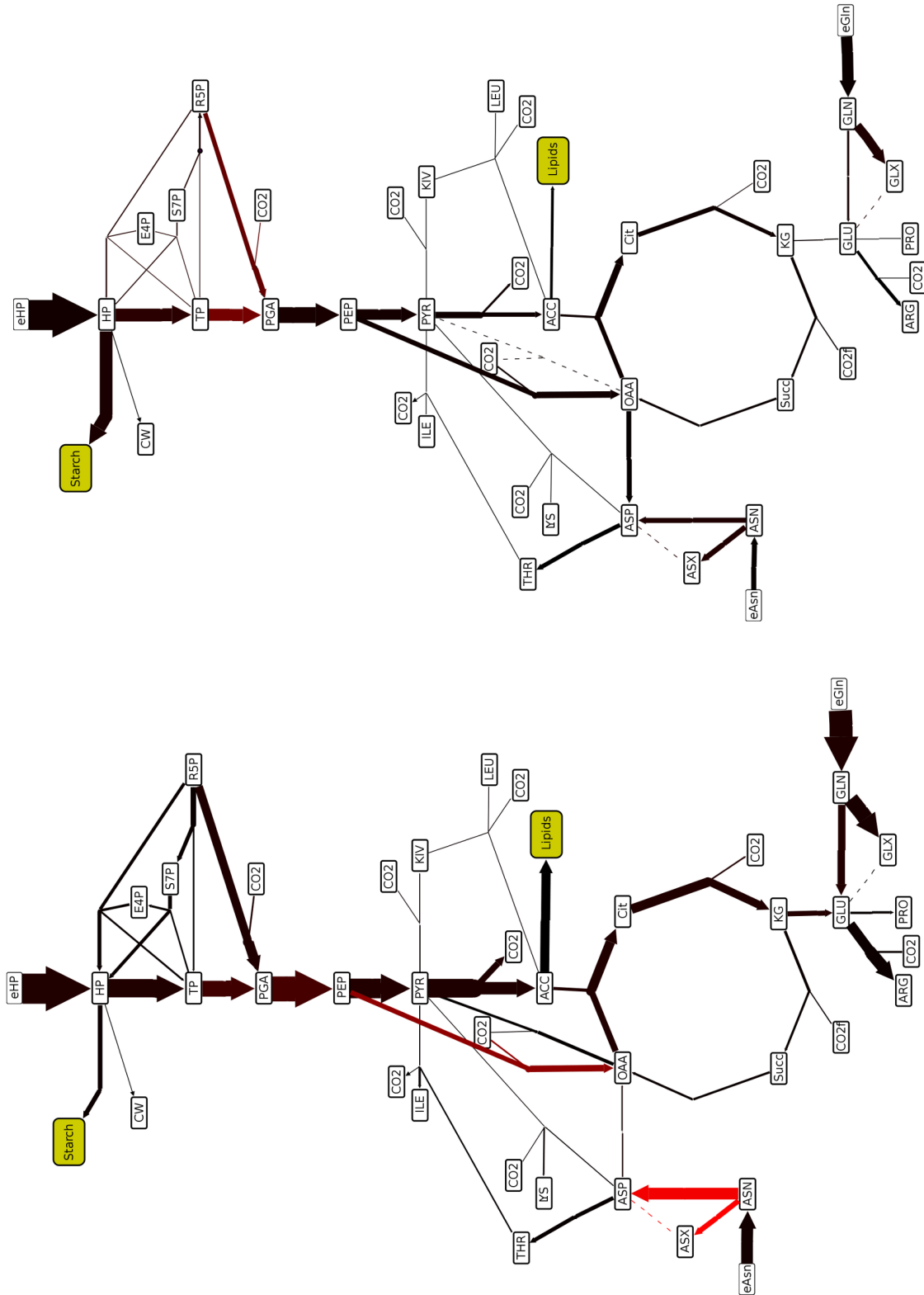


Figure 1.1: Flux maps of two different conditions, generated by FLUXMAP.

2 Installation

1. Download and install VANTED 2.0 (if not yet installed) from <http://vanted.org/>
2. Download FLUXMAP Add-on from <http://vanted.org/fluxmap/>
3. Start VANTED
4. Drag'n Drop the **FluxMap.jar** onto the VANTED frame or manually install FLUXMAP:
 - Click “Install / Configure Add-ons” in side panel “Help” → “Settings” of VANTED
 - Click Install Add-on
 - Choose **FluxMap.jar** as add-on to be installed
 - Click Install and OK
5. Download the flux data template from side panel “Experiments” → “Data Input Templates” by clicking at button “Flux Data” and saving the Excel file anywhere (e.g. on your desktop). This template is already filled with some artificial exemplary data.
6. Download and install the GRAPHVIZ-library from <http://www.graphviz.org/>, if you need an automatic layouter (please do not alter the name of the folder). See the installation instructions there.

3 Data Handling

3.1 Data Input

Simulated or measured flux data solely enters the system via a structured Excel-template (see Fig. 3.1). The template can be found in VANTED in the tab “Experiments” → “Data Input Templates”. Four parts of it concern experimental setup, such as experiment annotation, involved conditions, taken samples, and substance weights. Latter enables to model C- or N-fluxes by assigning a weighting factor to each substance (e.g. the number of C-atoms). The flux data itself is entered as a reaction formula and the corresponding flux measurement:

1. **Experiment:** annotation is specified by name, start date and coordinator (all string) of the experiment
2. **Conditions:** are specified by species, genotype, treatment (all string), and a unique ID (integer)
3. **Samples:** are specified by time point and time unit (integer and string respectively)
4. **Weights:** to represent any flux type (such as mass-, C- or N-fluxes) weights are specified by pairs [name,value] (case-insensitive string and float). In example, for C-fluxes the weight of CO₂ would be 1 and the weight for O₂ would be 0. All non-specified substance weights are assumed to be 1.

The flux data itself is entered via the other parts:

5. **Reactions** are specified by a formula and a reaction name (both string). The formula consist of two lists of reactant and product substances respectively. Substances are separated by a “ + ” (note the space characters), whereas both lists are separated by a reversible (“<==>”) or irreversible (“==>” or “<==”) reaction arrow. Substances itself are represented by a name or as a pair [stoichiometry,name] (float value and string, separated by a space character). Stoichiometry is explicitly allowed to be non-integer number and assumed to be 1, if not specified. Some examples:

```
“A ==> B + B” (equal to “1 A ==> 2 B”)
“A + 0.5 B <==> C”
“A <==> A_ext”
```

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		Internal Info			
Experiment		V1.0F		Substance weights	
Start of Experiment (Date)	18/01/11			name	eHP
Remark*				weight	HP
Experiment Name (ID)	Legum Fluxes				6
Coordinator	Björn Junker				
Sequence-Name*					
Conditions		1	2		
Species		Medicago	Pea		
Genotype		A17	Chiffel		
Treatment					
Measurements		Conditions	1	quality	2
		Time			quality
		Unit (Time)			
Reaction	Reactionname	Unit			
eHP ==> HP	uptH	relative flux	32.16560	3.201312032	32.1656 1.75137
eGln ==> Gln	uptGln	relative flux	29.524	2.952	14.446 0.997
eAsn ==> Asn	uptAsn	relative flux	18.754	1.888	8.131 0.598
HP ==> TP	vALDO	relative flux	19.714	3.203	13.709 4.187
TP ==> Gyc	vGLYC	relative flux	1.07	0.000	0.326 0.000
TP ==> PGA	vGAPDH	relative flux	33.84	6.341	25.569 11.493
PGA ==> PEP	vENOL	relative flux	53.555	6.867	33.754 2.391
PEP + CO2 ==> OAA	vPEPC	relative flux	10.367	4.305	10.455 1.618
OAA ==> Pyr + CO2	vME	relative flux	5.441	0.612	0.003 1.091

Figure 3.1: Excerpt of the data template. A user has to specify some experimental metadata (1), the conditions (2), the substance weights (3), the samples (4), the reaction-equations (5), and the flux values including quality information (6).

6. **Measurements** are specified as float values for each pair [condition, sample]. For each reaction the measurements have to be specified by indicating the condition using the condition id and time together with time unit. Optionally it is possible to add quality information, which may be standard error or any other confidence information, specified as a float value in a subsequent column titled “quality”.

The template enables users to enter structured data (in terms of conditions and samples) in a biochemical plausible notation. Possible spelling and other input errors are reported to the user during import or may be found using flux balance validation described in Sec. 3.4. Please do not add or remove columns and rows, as this may disturb the interpretation of the template.

To load the template into VANTED just Drag’n Drop the file onto the VANTED frame or use the button “Load Dataset” in the “Experiments” tab and choose the template file. FLUXMAP parses the template file and automatically builds up a special VANTED dataset for mapping experimental data onto edges.

3.2 How to create a network

- download from MetaCrop: go to tab “Pathways” and select the appropriate pathway
- download from KEGG: go to KEGG homepage, navigate to your pathway and press “Download KGML file” and load this file in VANTED
- load any SBML file or manually create a network
 1. fill flux template and load in VANTED
 2. map dataset onto empty graph and open Flux-Dialog
 3. click on “Split frequent nodes” and specify the substances you want to clone
 4. use DOT-layout to get a good automatic layout
 5. manually relayout nodes and edges until sufficient layout is achieved
 6. remember that this procedure needs about 1 hour, but usually is done just once in the beginning and used further on for visualising flux maps

Hint: an example flux map can be found in the “Example” tab of VANTED in the folder “Flux Data”.

3.3 Mapping and Network Representation

A network may be manually edited or loaded into the system from GML-, GraphML-, SBML-, and SIF-files beforehand. As VANTED does not support hypergraphs, a binary graph representation of the graph has to be generated by adding a reaction node for each reaction (labelled with the reaction name) and connecting all metabolites participating in this reaction with edges to or from this node. The dataset is automatically split by the parser in a similar way, respecting the stoichiometry of the reactant, the flux measurement value of the reaction, and the substance weights, thereby supporting the user in this tedious task. The resulting dataset can be mapped onto any graph using the respective VANTED-button in such a way, that each substance is represented by a node with a unique label and all flux data is mapped to edges between these nodes. Non-existing edges and nodes in the graph can be automatically added using the VANTED mapping dialog. It is even possible to map the data onto an empty network in order to automatically build up the binary network representation of the loaded dataset. In this case it is useful to manually or automatically (using the dialog) layout the network afterwards. Reversible reactions will have also an arrowhead. Edge direction will be adjusted according to the reactions in the template.

3.4 Balance Validation

Once mapped onto a network, the flux balance can be checked: For balanced flux distributions, the sum of all ingoing fluxes into a reaction node is equal to the sum of

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all outgoing fluxes, which should always be the case for real data (respecting a certain measurement error). This balance can be checked using the “Validate” button in the dialog (see Fig. 3.2). If needed, also the tolerance threshold for measurement errors may be adapted using the spinner next to the button. All unbalanced reactions will be reported to the user, thereby helping users to track down typographical errors, such as misspelled substance names (“*Sucr*” instead of “*Suc*”), wrong stoichiometry (“*2Suc*” instead of “*2 Suc*”), missing stoichiometry, and missing reactants and reactions. Such errors should be corrected in the template and the data import and mapping procedure to be repeated, until all fluxes are balanced.

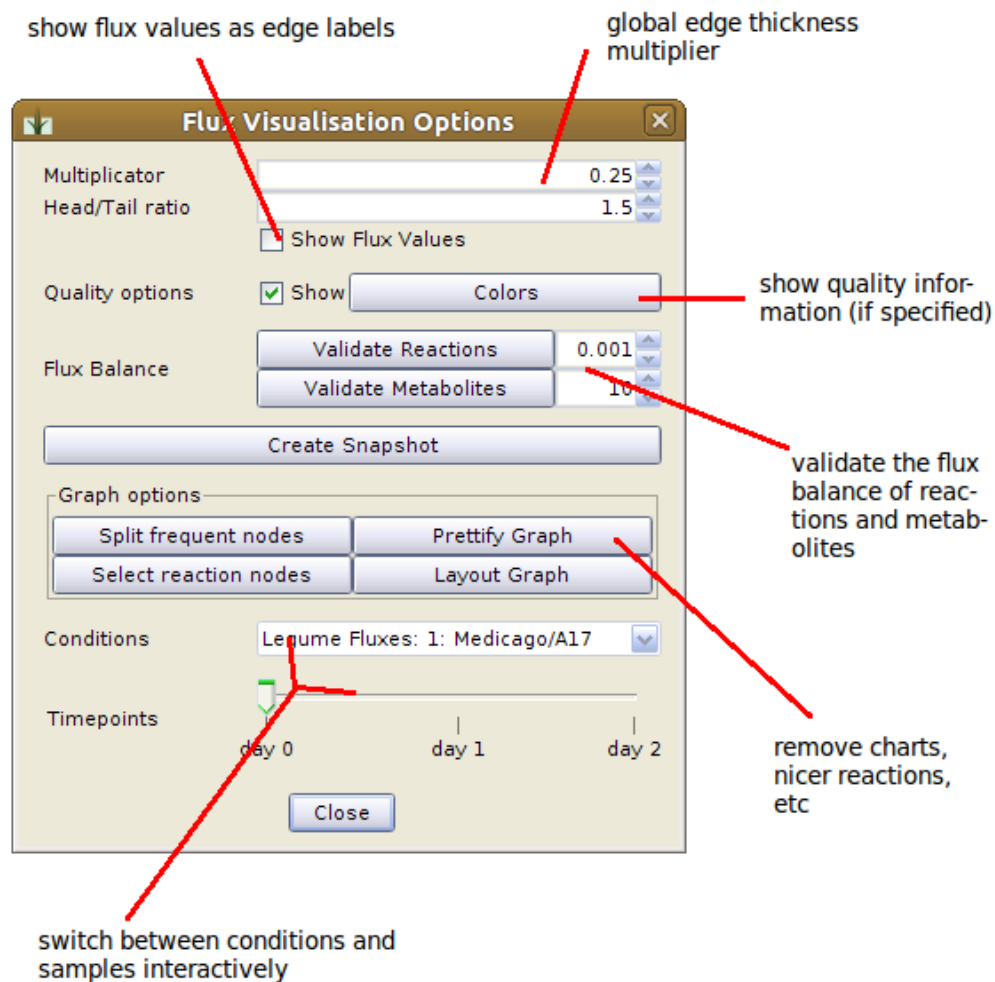


Figure 3.2: The dialog enabling users to modify flux map properties and flux visualization. It can be summoned by selecting menu entry "Mapping" → "Flux Visualization"

4 Visualization and Interaction

The calculation of edge thickness is triggered by executing the “Mapping” → “Flux Visualization” menu entry. This will open a dialog enabling users to interact with the flux map and modifying flux visualization parameters (see Fig. 3.2). Each parameter modification triggers a redraw of the flux map. Edges without flux data will be drawn in gray.

4.1 Visualization Parameters

Different controls enable to fine-tune many different visualization parameters. Two resulting and exemplary visualization styles can be seen in Fig. 4.1.

- **Multiplicator:** change the global edge thickness in order to adapt the edge thickness
- **Head/Tail Ratio:** change relative size of edge head to edge tail
- **Quality Options:** option to visualize the quality information (if specified) using color graduation
- **Prettify Graph:** remove unwanted charting and edge bends and select different visualization styles for reaction nodes, e. g. invisible, small, rounded or normal nodes with or without labels

4.2 Interaction

4.2.1 Exploration of Conditions and Samples

As flux data can be structured by conditions and samples, users want to interactively switch between these experimental factors. This is possible by using the combo box (for all conditions) and/or the slider (for all time points), which may then be used to explore and visually compare the corresponding flux maps. The appearance of this controls depends on your flux dataset (see also Fig. 3.2).

4.2.2 Layouting and Node Splitting

A proper network layout is a crucial step in understanding the flow of substances through the map. We tackle these requirements by utilizing the DOT-layout functionality of the GRAPHVIZ-library, which provides automated high-quality layout of biological networks.

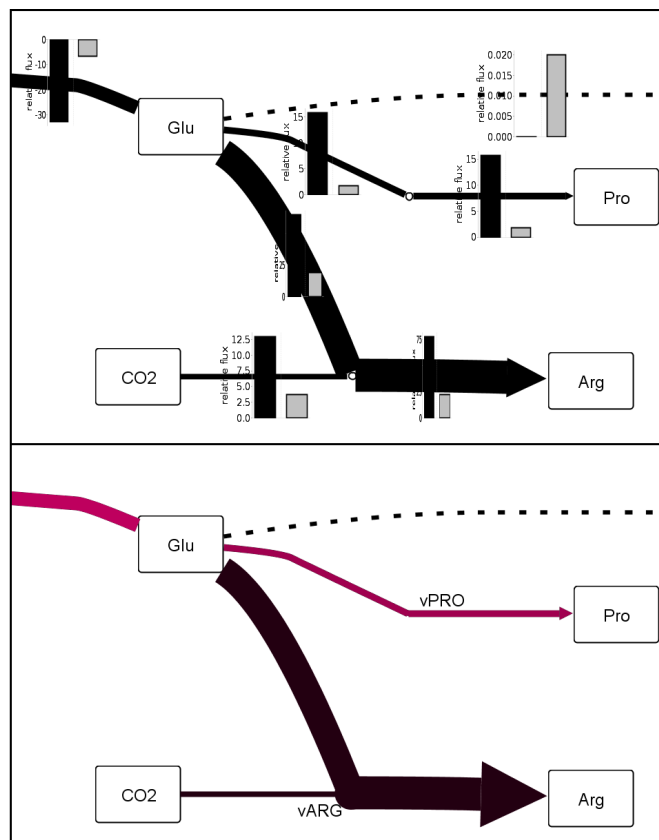


Figure 4.1: Two screenshots of the very same flux map with two different visualization styles. Top: Bar Charts indicating the flux values for different conditions (black bar represents the shown flux distribution), reaction node is represented by a small node and the arrowhead/-tail ratio was decreased. Bottom: red color indicates the quality information and the reaction nodes are round circles, realizing appealing flux visualization.

Press the “Layout graph” button to execute such layout. If you see a dialog indicating that DOT is not installed, please follow the sixth step of the installation instructions in Chapter 2. Other automatic layouts are also possible using layout algorithms from the “Layout” tab of VANTED. Direct editing additionally enables users to manually layout or further improve the existing layout.

Especially in metabolic networks some substances occur frequently in different reactions inside a flux map, such as ATP or CO₂. This leads to a large degree of interconnection, resulting in non-optimal network layout and cluttered visualization of the network. By clicking on “Split frequent nodes” in the dialog a user selects such nodes based on the degree of interconnection. These nodes are split into a number of cloned nodes, one for each reaction. Subsequently a proper layout can be realized and many edge crossings prevented.

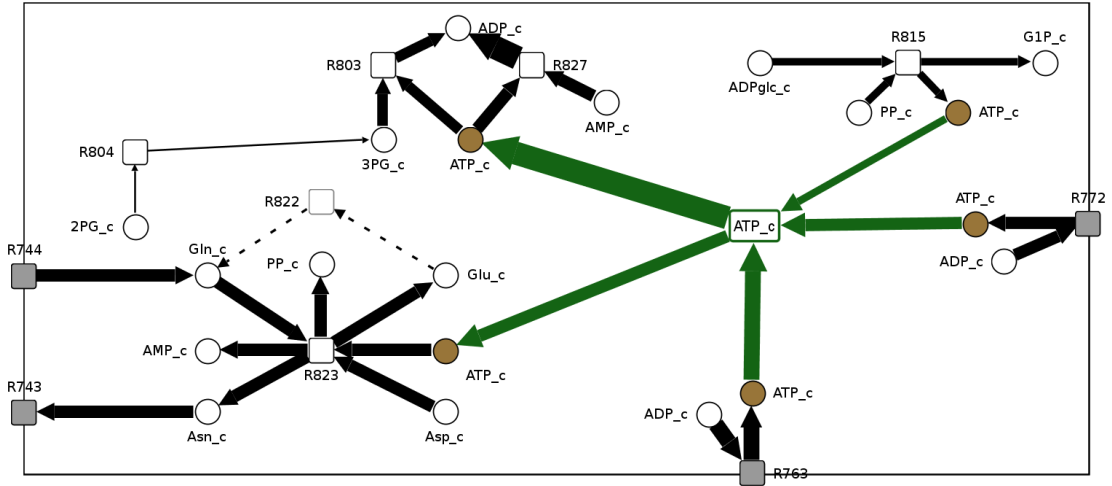


Figure 4.2: Part of a flux map, in which the widely distributed substance “ATP_c” was temporarily connected together (green elements).

4.2.3 Interactive exploration of scattered nodes

A negative effect of node splitting is that it gets hard to visually catch the global flux distribution through such nodes, especially if the map is large and the cloned nodes are distributed throughout the whole flux map. We tackle these problem by enabling users to temporarily connect these nodes again. This is achieved by selecting any number of substance nodes, right-clicking to bring up the context menu, and choosing “Node” → “Connect Nodes”. Thereby temporary all nodes with the same label are connected together (see green fluxes in Fig. 4.2).