Visual FTBL Generator: Visual Generation Tool for 13C-FLUX File

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HIGHLIGHTS

- The first web server for virtually generating FTBL file with high accuracy.
- Fast and easy converting FTBL files to metabolic networks.

Abstract: \textsuperscript{13}C metabolic flux analysis (\textsuperscript{13}C-MFA) has achieved increasing significance in quantitative metabolic system analysis in recent years. In \textsuperscript{13}C metabolic flux analysis, \textsuperscript{13}C-FLUX software is a major analytical tool. The software’s input script is primarily expressed in textual form without visual presentation of the structure of the entire metabolic network, thus error-prone in manual input. To solve this problem, we have developed a visual FTBL generator (VFG, available at http://47.100.98.220/vfg/index.jsp in a Google or Firefox browser) for MFA that eliminates the tedious, error-prone text entry mode and provides a user-friendly graphical interface and simple visual reaction generation functions.

Keywords: metabolic network; visualization; 13C-FLUX; database.
INTRODUCTION

The $^{13}\text{C}$ metabolic flux analysis ($^{13}\text{C}$-MFA) method is currently one of the most extensively employed tools for the quantitative study of the metabolic system [1]. This is a carbon labelling experiment (CLE)-based metabolic flux determination method [2].

$^{13}\text{C}$-MFA is capable of obtaining more information with a wider application range than a pure metrological metabolic flux analysis [1]. It could obtain the exchange flux of a metabolic pathway and does not depend on physiological assumptions, with the exception of a steady-state hypothesis [2,3]; thus, it applies to various experimental conditions. As it applies the experimental data acquired by mass spectrometry (MS) and nuclear magnetic resonance (NMR)[4,6,7], $^{13}\text{C}$-MFA is capable of solving problems that metrological methods cannot solve, such as metabolic loops and parallel reactions [1,3]. Therefore, studies of $^{13}\text{C}$-MFA are highly significant to the quantitative study of the metabolic system.

$^{13}\text{C}$-FLUX is a console-based metabolic flux analysis software that supports $^{13}\text{C}$-MFA modelling, can utilize a variety of experimental data, has high simulation efficiency, powerful functions of flux estimation and error analysis, and supports multiple users [1,8]. However, its description format of a metabolic model is rather complex, and the input script is primarily expressed in textual form, which causes a considerable number of disadvantages [1,5,8]. For example, $^{13}\text{C}$-MFA is unsuitable for beginners, is inconvenient for comparing, copying and editing different input files, and is unable to visually present the topological graph of the reactions in the entire metabolic network.

Considering these problems and the advantages of graphical tools [10,11], we developed a Visual FTBL generator (VFG) for the script entry of $^{13}\text{C}$-FLUX software (Figure 1), which uses Java + Jquery + EasyUI + MySQL + D3 as the development platform and Tomcat as the publishing server. VFG provides a user-friendly interface. The VFG can directly import the manually created $^{13}\text{C}$-FLUX scripts into a database and generate the network topological graph in real time, which renders it convenient for users to add, edit and copy the data.

![Figure 1. Architecture of the input script visualization tool for 13C-FLUX software.](image-url)
MATERIAL AND METHODS

Definitions and characteristics of VFG components

In the VFG, G: = <N, E> represents the metabolic network diagram, where N: = {R n, C n}, E: = {R e, P e}, N is the set of nodes, and E is the set of edges. Rn denotes the metabolites, which are represented by green circular nodes, and Cn denotes the enzyme node (i.e., the reaction conditions), which are represented by red circular nodes. The Re in the edge set represents the blue directed edge from the reactant node to the enzyme node, and Pe represents the directed edge from the enzyme node to the product node, as shown in Figure 2. In this manner, the reactants, reaction conditions and products of each metabolic reaction can be directly distinguished in the figure.

In the entire network structure (Figure 3), a user does not need to drag the components to make connections and edit the properties of reactants, enzymes, and metabolites. By entering the reactants, enzymes, and metabolites, a visualized graph can be automatically generated, which decreases the amount of time required by the user to construct a graph. The user can freely add multiple biochemical reactions and obtain a metabolic network with complex reaction relationships. In the generated metabolic network, the user can readily make additions, modifications, deletions and view the metabolic reaction data corresponding to the enzyme node. Check the drag radio button in the metabolic network graph and clicking on an enzyme, a user can highlight the relationship between the reactants and products of the current reaction. The entire metabolic network can be freely dragged, zoom in and out to enable users to view any node of the entire network.

The generating and editing functions of the VFG Biochemical reactions constitute the basic unit of the software. For example, the interface shown in Figure 4 corresponds to the following reaction: Reactant 1 + Reactant 2 <-> Product 1 + Product 2.

In the VFG’s editing environment. If it is a new user, the user needs to create a new panel in the Network Panel menu and enter an initial metabolic reaction. Then the user can directly entering reactants, enzymes, products, and their related stoichiometric coefficients through the Data Display option in the Data Operation menu. In the data display interface, when adding an enzyme, user needs to select the Add Reaction radio button and clicking on any red enzyme node in the canvas, the system will automatically display the corresponding data entry window to enable the user to enter experimental data that are related to a node and the sign of reaction reversibility. At last, the user can obtain the visual results shown in Figure 3, in which VFG automatically connects the nodes of a reactant, enzyme and product.

Each metabolic network graph within the VFG corresponds to a stoichiometric matrix model, whereas each graphic component also precisely corresponds to the specific data structure in the model. When editing the metabolic network, the VFG responds to the user’s actions in real time and synchronizes the revision of the upfront network topological graph and the internal data model. For example, once a reaction node on the graph is deleted, the node-linked edge that represents the relationship between the reaction condition and the product, as well as the corresponding nodes and all related constraint conditions, also need to be deleted.
The system will automatically check the validity of the user input and show messages in case of error for the user’s reference to ensure the correctness of the entered model.

**Figure 2.** Visual network components for $^{13}$C-FLUX text input

**Figure 3.** Main interface of the VFG operation
Modelling data entry for the visual FTBL generator

In addition to topological structure data, a $^{13}$C-FLUX script needs the related analytical data that are necessary for $^{13}$C modelling [1]. The following default data are added:

1. Atomic sequence label, in the following form of the FTBL file:
   
   $\text{A + B -> C + D}$  
   
   $\# \text{Abc} + \# \text{def} \rightarrow \# \text{Abef} + \# \text{cd}$

2. Stoichiometric constraints of flow rate

   The metabolic network that corresponds to the stoichiometric matrix contains the constraints on the flux rate. However, in the $^{13}$C-FLUX calculation model, additional stoichiometric restraints of EQUILITIES, FLUX_MEASUREMENTS or INEQUILITIES are also accepted. These constraints comprise a number of linear expressions that are consistent with the MathML norm, you can select the corresponding options in the Data Operation menu to enter the data.

3. $^{13}$C labelled data

   The $^{13}$C-FLUX calculation model also requires the $^{13}$C labelled data of the substrate, intermediate, and final product. The FTBL generator provides the entry interface for the corresponding data on the Data Operation menu page, which enables the input of the isotopomer score (LABEL_INPUT), the NMR detection limits of the metabolic intermediate and the product (PEAK_MEASUREMENTS), the mass isotopomer score (MASS_SPECTROMETRY), and any given isotopomer group (LABEL_MEASUREMENTS). Figure 5 shows the data entry window for these data, in which the user selects the module that requires data entry and adds values for various data when necessary. The operation is rather simple and is similar to entering data into Excel.
**Import and export of FTBL scripts**

The VFG provides one-click import and export functions and can generate the corresponding network topological graph in real time by importing the existing FTBL script file. The VFG can generate corresponding FTBL script from the metabolic network model and the $^{13}$C labelling data. If users want to import the existing FTBL script file, they must ensure the file name to upload is unique. If the file name has already existed in the system as a panel name, the file is invalid, or must be deleted from Panel Delete Menu. In the function of export the FTBL script file, users can select the panel name which are to be exported, then click the create FTBL file button and export it to your computer.

The software separates the metabolic network model and the storage of $^{13}$C labelling data. The former primarily includes the topological structure, reaction reversibility and atomic transfer information. This information is structured data that can be shared in different experiments and stored in str format, whereas the remaining reaction flux restrictions and $^{13}$C labelling data are inferred from specific experiment, which is saved in exp format.

**RESULTS AND DISCUSSION**

**VFG-based generation of FTBL file of the central $^{13}$C metabolism**

For most researchers, especially beginners, the traditional method for entering a $^{13}$C-FLUX script troublesome (Figure 6). In VFG, single reactions can be graphically linked to form a visual metabolic network graph that enables the user to view the relationship and changes for the reactants, enzymes and products in the entire metabolic network.

The central carbon metabolic network generated by the VFG is shown in Figure 7, which primarily includes the glycolytic pathway, pentose phosphate pathway, tricarboxylic acid cycle, glyoxylateshunt, and anaplerotic pathway and could be used to generate the corresponding FTBL files via the VFG.
The comparison between the VFG model and the FTBL file shows that the visualized input of the VFG can generate FTBL files that fully satisfy the requirements.

![Table showing the comparison between VFG and FTBL](image1)

**Figure 6.** $^{13}$C-FLUX script files of carbon metabolism biochemical reaction

![Visual metabolic network of carbon metabolism biochemical reaction](image2)

**Figure 7.** Visual metabolic network of carbon metabolism biochemical reaction

**Comparison between VFG entry and manual entry**

To compare the efficiency difference between VFG and manual input, we selected 32 groups of a central carbon metabolic network (including photosynthesis) and $^{13}$C-labelling data of ten species from 20 published papers as the target, which were entered by six students (in two groups) to compare the FTBL files via the two methods. The results indicated that the manual input completed 3 data sets at an error rate of 16.5% within one hour, whereas the VFG entry completed 4 data sets at an error rate of 5.6% within one hour. Within five hours, the manual input completed 17 data sets at an error rate of 21.7%, whereas the VFG completed 24 data sets at an error rate of 10.3% (Table 1).
Table 1. The efficiency difference between VFG and manual input

<table>
<thead>
<tr>
<th>Entry mode</th>
<th>students</th>
<th>Spend time</th>
<th>Amount of completion</th>
<th>error rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>manual input</td>
<td>3</td>
<td>1 hours</td>
<td>3</td>
<td>16.5%</td>
</tr>
<tr>
<td>VFG</td>
<td>3</td>
<td>1 hours</td>
<td>4</td>
<td>5.6%</td>
</tr>
<tr>
<td>manual input</td>
<td>3</td>
<td>5 hours</td>
<td>17</td>
<td>21.7%</td>
</tr>
<tr>
<td>VFG</td>
<td>3</td>
<td>5 hours</td>
<td>24</td>
<td>10.3%</td>
</tr>
</tbody>
</table>

These results indicate that the visual data input, coupled with the separation of the structure data and the labelling data, increases the scalability and compatibility of 13C-MFA data input.

By creating a new canvas and entering the reactants, reaction conditions and metabolites, a metabolic network reaction diagram can be generated in real time with editing the data by clicking on the reaction conditions. The VFG serves as a "what you see is what you get" (WYSIWYG) editor, and the entry of a reaction can generate a reaction diagram, which renders it convenient for editing the input data in a visualized metabolic network topological graph. The VFG also enables other property data, with the exception of the biochemical reactions, to be added to a web page and is capable of generating the required 13C-FLUX input scripts from the finished metabolic network topological graph and other analytical data.

CONCLUSION

Currently, the input script of the 13C-FLUX analysis software is primarily expressed in textual form, is error-prone in manual entry, and is unable to visually present the structure of the entire metabolic network. To solve these problems, we developed the VFG for 13C-MFA that eliminates the tedious, error-prone text entry mode and provides a user-friendly graphical interface and ready-to-use visualized reaction generation functions that are capable of generating the text file required by the 13C-FLUX analysis software with only one click. The VFG can facilitate the copy, transfer, modification and expansion of script file data in large-scale high-throughput metabolic flux analyses.

For visualization of metabolic network for 13C-MFA, a famous powerful visualization tool is "Omix". In Omix, the visualization of data coming from experiment or simulation is completely performed by the software user realized in concise OVL scripts [12]. Different from Omix, VFG is a specialized input script visualization generation tool for 13C-FLUX software. It is easy in understanding and operating. VFG only needs inputting the reactants, enzymes, and metabolites. A visualized graph can be generated automatically. At last, VFG divides the data structure for 13C-FLUX optimization into structure type data and analytical type data. This classification is useful in data consistency and standardization, and might be used in other fields.

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