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Interpretation and Integration of 13C-Fluxomics Data

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Abstract

The fluxome, based on 13C flux, is a newly developed important aspect of cellular science, and it is both the final read-out of the physiological state of cells and an extension of both the transcriptome and proteome. This article records the important methods in the technical development of 13C-fluxomics, reviews the significance and progress in the interpretation and integration of fluxomics data, and then explores several directions of vital importance for the interpretation and integration of 13C-fluxomics.

Keywords: Fluxomics; Metabolic flux analysis; Systems biology; Data mining; Integration model

Abbreviations

HSQC: Heteronuclear Single Quantum Correlation; 2D COSY: 2 Dimensional (2D) Correlational Spectroscopy (COSY); TBDMS: Tert-Butyldimethylsilyl; GC-MS: Gas Chromatography Mass Spectrometry; LC-MS: Liquid Chromatography-Mass Spectrometry; DNP: Dynamic Nuclear Polarization, EMU: Elementary Metabolite Unit; NMR: Nuclear Magnetic Resonance

Introduction

Metabolism provides both the materials for the composition of cellular systems and an energy source for the operation of other systems; changes in metabolic components can be used as the initiating signal for certain cellular processes. Thus, the metabolic system is an essential element in reflecting the internal physiological state of cells. For the description of cellular metabolism, apart from the metabolome (qualitative and quantitative analysis of metabolic components), the quantitative analysis of the mutual conversion between metabolic components is also of paramount importance. This analysis method is known as fluxomics.

The 13C fluxomics-based detection method mainly depends on the fact that, as far as cell substrates labeled with 13C are concerned, the intracellular flux determines the 13C distribution state of metabolites. This methodology has gradually developed and matured over the past 20 years. Its mathematical framework is based on the concept of the atom mapping matrix proposed by Zupke et al. and the concept of an isotopomer Schmidt matrix proposed by Schmidt et al. [1,2]. The concept of a cumomer introduced by Wiechert et al. has also been adopted to simplify the computational complexity of the isotopomer [3] and develop the most widely used software 13CFlux [4]. A flux estimation framework has recently been proposed and developed with regard to dynamic 13C labeling [5]. Antoniewicz et al. proposed a computational framework for elementary metabolite units, which has greatly reduced the amount of computation flux [6]. The method of metabolism flux proportional analysis developed by Sauer and Yang et al. has greatly reduced the probability of an invalid solution being identified and improved the accuracy of metabolic fluxomics, due to the introduction of 13C labeled spectra-based analytical constraints in the flux estimation process [7,8].

As far as 13C labeled spectral detection methods are concerned, the 2D HSQC proposed by Szyperski et al. has become a widely used method, as it can detect the distribution state of 13C-13C. The detection of amino acid TBDMS derivatives by GC-MS, developed by Dauner et al., has played a major role in recent literature [9,10]. Recently, Rühl et al. and Young et al. have achieved good results by using LC-MS or LC-MS/MS to detect the state of 13C labeling [11,12].

Based on their work and the increasingly wide use of 13C metabolic flux methods in recent years, more and more metabolic flux data have been accumulated, with fluxomics itself emerging as a new way to describe cell systems. Thus, fluxomics has gradually matured into a new -omics technology that can be mentioned in the same breath with proteomics and metabolomics. What follows then is that the interpretation of metabolic fluxomics data is becoming more and more important. The interpretation of fluxomics data currently relies predominantly on Genome-Scale Metabolic Network Models (GSMR) [13]. Burgard et al., relying on GSMR and a Flux Balance Analysis framework, developed a method of predicting the biological significance of specific metabolic flux [14]. The method uses bi-level linear programming and can identify an objective function that leads to a specific metabolic flux distribution. Cell growth under either aerobic or anaerobic conditions has the same metabolic objective, but different metabolic fluxes due to the differences in input conditions. Schuetz et al. projected metabolic flux data from the Sauer laboratory onto a three-dimensional flux space, and using the multi-objective optimization method, they found that these flux data were distributed on the Pareto-front produced by several different objective functions, indicating that the evolution of the flux state was achieved by a shift among different objectives [15]. By combining metabolic flux data, GSMR and multi-objective optimization, we found that, in the case of oxidative stress, the central carbon metabolism of *E. coli* could transit from the normal state into a suboptimal state, resulting in more NADPH to fight against oxygen free radicals [16].

Corresponding with the interpretation of fluxomics data is the integration of fluxomics data with other -omics data to provide greater biological knowledge. Progress in this area has been slow in recent years or to put it in another way, has not yet attracted enough attention from relevant researchers. However, the number of metabolic fluxomic data

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sets is predicted to grow rapidly in the future. There will be growing demands for the integration of metabolic flux data, other -omics data and physiological data, which will further require the corresponding development of large-scale storage, retrieval and analysis tools. Therefore, we have recently collected more than 100 articles about fluxomics and mined them for approximately 600 pieces of metabolic flux data and constructed a visual database of metabolic flux. At the same time, we combined metabolic network alignment methods with flux data characteristics to design a metabolic flux data comparison scoring method. This method provided the basis for the retrieval and analysis of metabolic flux which we then incorporated into our website, representing a step towards the large-scale storage, retrieval and analysis of metabolic fluxomics data [17].

As the end read out of DNA encoded information, metabolic flux not only has a key role in describing the physiological state of cells, but also is an intermediate indicator between biomedical and bio-manufacturing industries that cannot be overlooked in the future. Therefore, there are many issues in fluxomics that require more energy to solve. For example, given that the method of fluxomics is now limited to the central carbon metabolism pathway, how then to extend it to other metabolic pathways, or even on a genome-wide scale? A method referred to as rapid dissolution DNP, which yields molecular probes in liquid samples to enhance the NMR detectable signal to a significantly high level, and its revision version might be considered as a promising technology to enlarge the scope of *in vivo* flux quantification [18]. How can we integrate fluxomics with metabolomics, proteomics and transcriptomics? A recent progress was a whole-cell computational model including all of biomolecular components and their interactions [19]. In addition to development of stand-alone software systems (Table 1) [20-26], the building of open platform for public use should be emphasized considering that the huge demand of simulation or decomposition calculation of metabolism systems has exceeds the limitation of current computer memory [27]. These are all issues that await more in-depth studies.

Software	Description
FiatFlux [26]	a software for metabolic flux analysis from labeled mass spectrometric data with flux ratio constraints
Flux-P [24]	an automate and standardized workflow of 13C-based metabolic flux analysis in the Bio-jETI workflow framework
iMS2Flux [23]	a high–throughput pipline for isotope labeled mass spectrometric data for metabolic flux analysis
INCA [20]	the first publicly available computational platform for isotopically non-stationary metabolic flux analysis
IsoDesign [22]	a software for optimized design of 13C-metabolic flux analysis experiments
OpenFLUX [25]	a software for metabolic flux analysis based on Elementary Metabolite Unit (EMU) framework
OpenMebius [21]	an open source software for isotopically nonstationary metabolic flux analysis

Table 1: Description of software used.

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