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Tandem Mass Spectrometry: A New Platform for Fluxomics

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Recent advances in various 'omics' technologies enable quantitative monitoring of the biological states of an organism in a high-throughput manner, and thus allows determination of the variations between different biological states on a genomic scale. Transcriptomics measures mRNA transcript levels; proteomics quantifies protein abundance; metabolomics determines concentrations of small cellular metabolites; and fluxomics defines turnover rates of molecules over the metabolic network in a biological system. Among the "omics", fluxomics is the ultimate manifestation of the phenotype and function of an organism as metabolic fluxes underlie all the biological activities including abundance of molecules and cellular signaling, regulation and transport. More importantly, fluxomics provides pathway activity information, which discerns the contribution of each pathway to the overall metabolite concentration and thus helps the understanding of complex metabolism in an organism. This information simply does not exist in concentration-based datasets. However, compared with transcriptomics, proteomics and metablomics, technologies used for fluxomics are less mature and not high-throughput because fluxomics requires isotope labeling experiments and mathematical modeling and both present difficulties. The major barriers are (i) insufficient measurements of isotopic labeling information in the metabolites for mathematical modeling for solving metabolic fluxes; and (ii) lack of appropriate computational algorithms for simulating the isotopic labeling data to find the fluxes with precision and reliability.

For the isotope labeling experiment, stable isotope labeled substrates (usually ^{13}C) are fed into the biological system, the isotope tracers are then distributed over the metabolic pathways and incorporated into the intracellular metabolites via the administration of biochemical reactions. The isotopic pattern and abundance of isotopic isomers (isotopomers) within a metabolite pool can be measured. At present, there are two established methods for measuring isotopomers for fluxomics, i.e., nuclear magnetic resonance (NMR) and mass spectrometry (MS). The NMR technique provides detailed positional isotopic labeling information (the positions of isotope labels in a molecule), which are useful for resolving the mathematical model for fluxes; however, NMR has low sensitivity (in nanomolar detection range for ^{1}H NMR) and is difficult to quantify isotopomers in low concentration metabolites. The MS, on the other hand, provides sensitive detection of molecules (in femtomolar detection range) and fractional enrichment of mass isotopomers (grouped positional isotopomers); however, it does not distinguish the positional labeling within the mass isotopomer and therefore limits the resolution of the mathematical model. These limitations severely limit the scope of fluxomics, e.g., most of the flux analysis studies have been limited to the central metabolism, and thus there is a clear need for new techniques that can address these issues. Development of tandem MS (MS/MS) technique offers the opportunity of gaining positional labeling information by fragmenting the parent molecular ion and measuring the mass distribution of the daughter ions, which is equivalent to NMR while maintains the sensitivity of MS, and thus has the potential for elucidating complex metabolic pathways. For example, if suitable daughter fragments can be obtained for fatty acids, it should be possible to perform detailed studies of fatty acid metabolism, which has been a difficult problem to address using traditional NMR and MS techniques.

Studies about application of tandem MS in ^{13}C isotope tracer experiment for fluxomics have recently gained attention [1]. Pioneering work by Jeffrey et al. [2] first demonstrated that the positional labeling information of ^{13}C-glutamate extracted from heart tissue per fused with various ^{13}C substrates could be generated using gas chromatography coupled tandem MS (GC-MS/MS) and the GC-MS/MS data could be used to derive metabolic fluxes with similar accuracy and precision to those obtained using NMR and significantly better than those using full-scan GC-MS. Later, Kiefer et al. [3] reported the determination of carbon labeling distribution of intracellular metabolites for fluxomics using liquid chromatography tandem MS (LC-MS/MS) and was able to determine the natural abundances of the carbon fractions *m* and *m+1* from six phosphorylated metabolites from glycolysis and pentose phosphate pathway in *E. coli* with high accuracy. More recently, Ruhl et al. [4] exploited the measurement of the ^{13}C patterns of totally 27 intact and 19 fragmented metabolites from the central metabolic pathways in *B. subtilis* using LC-MS/MS, and the metabolic fluxes calculated using the LC-MS/MS data showed higher precision than those performed using solely full-scan LC-MS data [4]. Most importantly, these research demonstrated that tandem MS, especially LC-MS/MS that does not require chemical derivation like GC-MS/MS, can provide direct measurements of large sets of isotopic labeling patterns from intracellular intermediates and thus allow resolving fluxes for dynamic systems (non-steady state) and large-scale complex metabolic pathways, which are infeasible with the traditional NMR and MS based fluxomics methods as those rely on indirect measurements of the abundant proteinogenic amino acids and thus require long labeling experimental time for steady state to allow the incorporation of isotopes into proteins and can only derive fluxes for the central metabolic pathways that have the key nodes linked with protein biosynthesis. Given the promising applications of tandem MS for fluxomics, however, the technical aspects for isotopomer measurements have to be stressed. First, the methods for metabolite extraction and separation need to be developed or optimized to increase the coverage of detection of various catalogues of metabolites considering the diversity of the physico-chemical properties of the metabolome in an organism. Second, the tandem MS instrumental parameters such as the collision energy for fragmentation, the resolution of mass filter and the number of multiple reaction monitoring (MRM) etc. need to be optimized to capture as many as possible informative daughter mass spectra that are useful for solving the mathematical model and minimize the acquisition of redundant daughter mass spectra that complicate the mathematical

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model for locating the position of labeled atoms [2,4]. For the computation of fluxes, a mathematical model that describes the relationship between the abundances of isotopomers and the fluxes, expressed as a system of isotopomer mass balance equations, need to be formulated. Different from the types of data by NMR and MS, the tandem MS data is more complicated and needs specific mathematical descriptions for mapping isotope atom transitions between the parent molecule and the daughter fragments. Choi and Antoniewicz have recently developed a general mathematical framework, called Tandem Mass Isotopomer Matrix, describing such parent-daughter carbon transitions in tandem MS, which can be incorporated into the mathematical model for simulating tandem MS data for fluxes from tandem MS measurements [1]. This work paved the way for the computational implementation of fluxes using tandem MS technique. However, the mathematical system is rather complicated and usually over-determined in terms of the unknown fluxes for the given isotopomer measurements, and involves a large-scale non-linear parameter fitting to minimize the difference between experimentally measured and computationally simulated isotopomer abundance data through iterative procedures. Resolving such a complex mathematical model could suffer from implementation complexity and convergence problems using the traditional Isotopomer Mapping Matrix (IMM) and Cumulative Isotopomer Balancing (Cumomer) algorithms. Recently, the Elementary Metabolite Units (EMU) approach was introduced that decomposed the metabolic network into EMUs and minimized the amount of information needed for simulation of target isotopomers and enabled significant improvement of the computational efficiency [5]. More recently, the Fluxomers method was developed, which introduced a new set of state variables that combined the fluxes and isotopomer abundances for simulating the isotopomer data and reduced the complexity and non-linearity of isotopomer balance equations and consequently improved the computational efficiency by avoiding simulating fluxes and isotopomer variables separately [6]. In the current implementation, the tandem MS transitions are simulated for metabolic flux analysis using the traditional algorithms in simple metabolic networks [1]. It is necessary to develop efficient and robust universal computational algorithms and software suitable for simulating tandem MS data for high-throughput fluxomics in large-scale complex metabolic networks. For example, incorporation of Tandem Mass Isotopomer Matrix framework into EMU or Fluxomers algorithms could be researched. The future algorithms will significantly accelerate the application of tandem MS for high resolution fluxomics in very large scale biological networks. In summary, tandem MS provides direct, high-throughput and sensitive determination of isotopic labeling information from intracellular metabolites for fluxomics studies. With the development of suitable and efficient computational algorithms, tandem MS based fluxomics will become an important tool widely used for elucidating large-scale complex metabolic networks in biological systems, which will have many applications in basic biological science, biotechnology and biomedicine.

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