



Stable Isotope Resolved Metabolomics Using NMR in Systems Biochemistry

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BIOCHEMISTRY

Metabolism is the fundamental function of living cells, and measuring metabolic state provides a dynamic picture of the cells or tissues and how they respond to external changes, such as disease or medical therapy. NMR is a highly versatile analytical method that can be used to solve a wide variety of biochemical problems (Rom et al., 2016). Despite its low sensitivity, its adaptability makes it an ideal instrument for evaluating biochemical dynamics both in vitro and in vivo, especially when combined with its isotope editing capabilities, which allow for easy determination of isotope distributions (Hampton 2017). These are essential for any flow analysis in living organisms. NMR spectroscopy is a potent and adaptable tool for determining the structure and shape of small and macromolecules in fluid or solid state. Furthermore, NMR approaches provide detailed information about molecular and internal dynamics over a wide variety of timescales by measuring rotational and translational diffusion coefficients and internal motions of molecules. Furthermore, NMR can be used in chemical dynamics to analyse mechanisms as well as flow measurements in vitro and in vivo (Rom et al., 2018). The isotope-selective detection and sensitivity of nuclear spin characteristics to the intra- and inter-molecular environment, as well as the robust and quantitative nature of NMR experiments, are crucial features for molecular structural and quantitative investigation. Because of these benefits, NMR was an early choice for metabolite profiling initiatives and a great companion for mass spectrometry (MS)-based metabolite profiling (Caldow et al., 2016). NMR analysis, for example, gives critical structural data such as functional groups, covalent bonds, and non-covalent interactions such as stereochemistry that are difficult to get using MS approaches. Metabolomics has emerged as a thriving subject for studying the functional biochemistry

of various organisms and model systems, as well as their reactions to changed circumstances and diseases. Although various mass spectrometry platforms are commonly used in metabolomics studies, the unique capabilities of NMR provide several advantages such as isotope-selective editing of complex mixtures, detailed positional isotopomer analysis for enriched metabolites, de novo structure determination of unknown metabolites (both unenriched and enriched), accurate quantification without the need for standards, and in situ analysis of pathway dynamics from cells to whole organisms. Although NMR is an innately precise quantitative approach for determining steady-state metabolite concentrations, fluctuations in metabolite quantities only convey half of the metabolic narrative (Heresco et al., 1999). This is due to the fact that not only are most intracellular metabolites maintained at relatively tight concentration ranges (homeostasis), but metabolic pathways are also densely coupled in a complex network of events. For example, the key roadways of glucose metabolism. Cells use endergonic anabolism to produce the precursors of biomass components such as nucleotides, lipids, and certain amino acids in order to multiply (Rom et al., 2017). Furthermore, in order to maintain homeostasis during proliferation, metabolites must be generated or acquired from the external medium. Complex metabolites, such as nucleotides, require many pathways to supply the carbon and nitrogen that form the product, and these processes are indeed complex.

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