



METHODOLOGY

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# Decoding the dynamics of cellular metabolism and the action of 3-bromopyruvate and 2-deoxyglucose using pulsed stable isotope-resolved metabolomics

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## Abstract

**Background:** Cellular metabolism is highly dynamic and continuously adjusts to the physiological program of the cell. The regulation of metabolism appears at all biological levels: (post-) transcriptional, (post-) translational, and allosteric. This regulatory information is expressed in the metabolome, but in a complex manner. To decode such complex information, new methods are needed in order to facilitate dynamic metabolic characterization at high resolution.

**Results:** Here, we describe pulsed stable isotope-resolved metabolomics (pSIRM) as a tool for the dynamic metabolic characterization of cellular metabolism. We have adapted gas chromatography-coupled mass spectrometric methods for metabolomic profiling and stable isotope-resolved metabolomics. In addition, we have improved robustness and reproducibility and implemented a strategy for the absolute quantification of metabolites.

**Conclusions:** By way of examples, we have applied this methodology to characterize central carbon metabolism of a panel of cancer cell lines and to determine the mode of metabolic inhibition of glycolytic inhibitors in times ranging from minutes to hours. Using pSIRM, we observed that 2-deoxyglucose is a metabolic inhibitor, but does not directly act on the glycolytic cascade.

**Keywords:** Stable isotope labeling, GC-MS, Cancer metabolism, Glycolysis, Metabolomics, 3-Bromopyruvate, 2-Deoxyglucose

## Background

Central metabolism is highly flexible and continuously adjusts to the physiological program of the cell, organ, and organism. In a healthy state, cellular metabolism is tightly regulated to guarantee physiological function and the efficient usage of available resources. However, the underlying mechanisms leading to metabolic dysfunction are often poorly understood. To gain deeper insights into the dynamics of metabolism, techniques enabling a quantitative and time-resolved analysis of the metabolome are needed to allow a detailed real-time view of carbon

routing through central carbon metabolism (CCM): glycolysis, pentose phosphate pathway (PPP), tricarboxylic acid cycle (TCA) cycle, and amino acid metabolism.

Metabolic flux is an expression of the activity of a network of enzymatic reactions. This network is influenced by several factors: the expression levels of the catalytic enzymes and the relative concentrations of substrates and products. In addition, allosteric regulation and post-translational modifications, including phosphorylation, ubiquitylation and, most recently recognized, acetylation, control metabolic activity. Metabolic flux cannot be inferred from transcript or protein levels of constituent enzymes, but rather requires direct measurement. Several techniques for metabolic flux analysis (MFA) have been established previously. Flux balance analysis (FBA) and stoichiometric MFA rely on

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# Metabolism 3 Bromopyruvate 2 Deoxyglucose Isotope Resolved Metabolomics

**Terry C. Jones**



### **Metabolism 3 Bromopyruvate 2 Deoxyglucose Isotope Resolved Metabolomics:**

*Decoding the Dynamics of Cellular Metabolism and the Action of 3-Bromopyruvate and 2-deoxyglucose Using Pulsed Stable Isotope-resolved Metabolomics* Mannheim Mannheim Press, 2015-12-15 This open access article by various authors presents key research on Decoding the dynamics of cellular metabolism and the action of 3 bromopyruvate and 2 deoxyglucose using pulsed stable isotope resolved metabolomics Cellular metabolism is highly dynamic and continuously adjusts to the physiological program of the cell The regulation of metabolism appears at all biological levels post transcriptional post translational and allosteric This regulatory information is expressed in the metabolome but in a complex manner To decode such complex information new methods are needed in order to facilitate dynamic metabolic characterization at high resolution Metabolism in Cancer Thorsten Cramer, Clemens A. Schmitt, 2016-08-24 This textbook presents concise chapters written by internationally respected experts on various important aspects of cancer associated metabolism offering a comprehensive overview of the central features of this exciting research field The discovery that tumor cells display characteristic alterations of metabolic pathways has significantly changed our understanding of cancer while the first description of tumor specific changes in cellular energetics was published more than 90 years ago the causal significance of this observation for the pathogenesis of cancer was only discovered in the post genome era The first 10 years of the twenty first century were characterized by rapid advances in our grasp of the functional role of cancer specific metabolism as well as the underlying molecular pathways Various unanticipated interrelations between metabolic alterations and cancer driving pathways were identified and currently await translation into diagnostic and therapeutic applications Yet the speed quantity and complexity of these new discoveries make it difficult for researchers to keep up to date with the latest developments an issue this book helps to remedy **The Warburg Effect Regulation Under Siege: The Intertwined Pathways in Health and Disease** Concetta Bubici, Salvatore Papa, 2019-10-17 Many cells including immune neuronal cancer and stem cells become dependent on aerobic glycolysis to escape apoptosis and accommodate their bioenergetics needs How this metabolic change also known as the Warburg effect is regulated remains largely unknown The Warburg effect has been widely investigated in cancer cells where it was first observed with the aim of decoding the molecular networks controlling its activation for therapeutic purposes This Research Topic aimed to discuss and review all the intracellular signaling regulating the Warburg effect in cancerous and normal non cancerous cells though original research articles mini reviews and reviews *Metabolomics and Its Impact on Health and Diseases* Veronica Ghini, Kathleen A. Stringer, Claudio Luchinat, 2023-03-03 This volume of the Handbook of Experimental Pharmacology which celebrated its 100th anniversary in 2019 addresses the rapidly growing and evolving field of metabolomics It has been compiled and designed to broaden and enrich your understanding as well as simplify a complicated picture of the diverse field of metabolomics This is accomplished by chapters from experts in the field on basic principles as well as reviews and updates of

analytical techniques The variety and different perspectives of the NMR approaches are described in the chapters By David Wishart Daniel Raftery and Ryan McKay while mass spectrometry advances are covered by Charles R Evans and Stefan Kempa This book also reflects the state of the art in the application of metabolomics to cell biology Marta Cascante and Ulrich Guenther and chapters that share insights into the application of metabolomics in various diseases Paola Turano and Claudio Luchinat Rachel S Kelly and Jessica Lasky Su Paige Lacy and Angela Rogers Relationships of metabolomics with drugs are highlighted by Robert Verpoorte natural products drug discovery by Oscar Millet and by Turano and Luchinat perspectives in precision medicine and by Daniel L Hertz drug induced peripheral neuropathy From the above list of diverse topics we believe this book has interdisciplinary appeal and scholars with an interest in the role of metabolomics in achieving precision medicine will find it of particular or special interest

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Weckwerth,2023-10-30 We are now entering the third decade of the 21st Century and especially in the last years the achievements made by scientists have been exceptional leading to major advancements in the fast growing field of Metabolomics Frontiers has organized a series of Research Topics to highlight the latest advancements in science in order to be at the forefront of science in different fields of research This editorial initiative of particular relevance led by Dr Wolfram Weckwerth Specialty Chief Editor of the Metabolomics section is focused on new insights novel developments current challenges latest discoveries recent advances and future perspectives in the field of Metabolomics

Sample Preparation in Metabolomics Julia Kuligowski,Guillermo Quintás,2021-04-07 Metabolomics is increasingly being used to explore the dynamic responses of living systems in biochemical research The complexity of the metabolome is outstanding requiring the use of complementary analytical platforms and methods for its quantitative or qualitative profiling In alignment with the selected analytical approach and the study aim sample collection and preparation are critical steps that must be carefully selected and optimized to generate high quality metabolomic data This book showcases some of the most recent developments in the field of sample preparation for metabolomics studies Novel technologies presented include electromembrane extraction of polar metabolites from plasma samples and guidelines for the preparation of biospecimens for the analysis with high resolution magic angle spinning nuclear magnetic resonance HR MAS NMR In the following chapters the spotlight is on sample preparation approaches that have been optimized for diverse bioanalytical applications including the analysis of cell lines bacteria single spheroids extracellular vesicles human milk plant natural products and forest trees

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