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代谢轮廓分析
研究方法与实验方案

Metabolic Profiling
Methods and Protocols

Thomas O. Metz

科学出版社

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科学出版社
北京

图字：01-2012-6960 号

This is an annotated version of
Metabolic Profiling: Methods and Protocols
Edited by Thomas O. Metz.

ISBN: 978-1-61737-984-0

图书在版编目(CIP)数据

代谢轮廓分析：研究方法与实验方案＝Metabolic Profiling: Methods and Protocols：英文 /（美）梅斯（Metz, T. O.）主编 . —北京：科学出版社，2013. 1
（实验室解决方案）
ISBN 978-7-03-035929-2
Ⅰ. ①代… Ⅱ. ①梅… Ⅲ. ①代谢—研究—英文 Ⅳ. ①Q493. 1
中国版本图书馆 CIP 数据核字（2012）第 259569 号

责任编辑：李小汀 田慎鹏 / 责任印制：钱玉芬
封面设计：耕者设计工作室

科学出版社 出版
北京东黄城根北街 16 号
邮政编码：100717
http://www. sciencep. com
双青印刷厂 印刷
科学出版社发行 各地新华书店经销
*
2013 年 1 月第 一 版 开本：787×1092 1/16
2013 年 1 月第一次印刷 印张：25 1/2
字数：600 000
定价：145. 00 元
（如有印装质量问题，我社负责调换）

《代谢轮廓分析：研究方法与实验方案》中文导读

代谢组学是系统生物学研究领域内的重要分支之一，其研究对象主要是特定生物体系内的各种代谢物。代谢组学概念的正式形成虽然不过数十年时间，但是其研究雏形最早可追溯到20世纪中期。的确如本书作者在序言中所提到的，现代代谢组学的形成与发展与早期药物分析和先天性代谢缺陷的诊断需求密不可分，虽然这种需求即使在当下仍然存在甚至还很迫切。

本书是《分子生物学方法》系列丛书中的一卷。全书共21章，依研究内容不同可大致分为如下几个部分。第一部分简要介绍了代谢轮廓分析的发展简史（第1章）；第二部分主要针对先天性代谢缺陷诊断而建立的适合血和尿中氨基酸（第2、4章）、肉碱类（第3章）、有机酸（第4章）以及核苷碱基类物质（第5章）分析的方法和流程；第三部分例举了人体内各种常见代谢物的分类检测方法和技术，涉及胆汁酸类（第6章）以及中心碳代谢中的糖酵解、三羧酸循环和磷酸戊糖途径代谢物（第7－9章）等；第四部分主要是针对不同样本和代谢物类型，对代谢组学领域内主流的两大分析技术——色谱（包括毛细管电泳）-质谱联用（第11－16章）和核磁共振（第19－20章）的应用分别给予概括性描述；第五部分则介绍了上述主流技术在药物代谢物检测领域的应用（第10、18章）；最后一部分简要探讨了色谱-质谱联用和核磁共振分析技术所得数据的处理方法和流程（第17、21章）。本书利用了近4/5的篇幅来介绍色谱-质谱联用技术，而且多以人源样本为对象，可见色谱-质谱联用技术在该领域内的独特优势。相对于核磁共振技术而言，色谱-质谱联用技术具有运行成本和维护费用低、检测灵敏度高等方面的优势。核磁共振技术的最大优势体现在无创性、可用于活体检测和检测通量方面。书中所涉及的内容有很强的实用性和针对性。本书中的所有章节都如同一份完整的实验记录或用户手册。任何有一定分析化学基础的读者都可以按照本书中所介绍的实验流程开展工作。更为可贵的是，作者在每章正文的后段将实验操作中的注意事项和易导致偏差或失误的地方都做了必要解释和说明，这无疑为致力于该领域研究的读者提供了难得参考。本书末尾还设有主题词索引，供读者快速寻找感兴趣的内容进行阅读。

代谢组学概念的形成虽然时间很短，但是由其衍生出的各种概念和学术争鸣层出不穷。甚至“代谢组学”本身也存在着实质所指并无本质区别的两个英文单词——metabolomics和metabonomics。一般地，多数学者将代谢组学研究对象限定在内源性化合物范围，因此药物代谢物分子的定性定量问题常被排除到代谢组学研究范围以外而隶属于药代动力学研究范畴。但是我们看到本书中作者并未刻意追求这种严格的区分，几个章节涉及了药物代谢物分析内容。这一方面反映了分析内源性化合物和药物代谢物在平台技术上的类似性，另一方面是出于代谢组学与药代分析在理念上不可分割的渊源。如果考虑到外源性的药物分子自进入人体或生物机体后都有一个吸收、分布、代谢和排泄过程的话，将进入机体内的药物代谢物分子纳入代谢组学研究范畴亦无原则性错误。换言之，这种争议或歧义仅仅是学科层面上的问题而非科学层面上的问题。事实上，准确界定内源性还是外源性本身也存在技术上和理论上的困难。比如说，无论食物中吸收而来的葡萄糖还

是自身糖原分解而来的葡萄糖，在血循环中呈现出的是完全无差异的理化和代谢特征。

就本书所述内容而言，以代谢轮廓分析（metabolic profiling）为题恰如其分。由于平台技术的限制，目前还无法获取任何一个物种的完整代谢组数据，所以任何代谢组学分析都是有限个代谢物的检测。但是代谢轮廓分析实则属于代谢组学（metabolomics）分析的一个侧面。诚如作者在序言中所指，相关的衍生概念还包括代谢靶标分析（targeted metabolite analysis）、代谢指纹分析（metabolic fingerprinting）等等。这些概念的区别更多地是出于所关注的或一次能检测、鉴定到的化合物的种类或数量，以及检测前对目的化合物的知晓程度而已，各衍生概念下的研究对象或内容的实质是一致的。读者还会发现，本书的第15、16章又出现了脂质组学（lipidomics）一词，而该术语仅仅是将所检测的对象限定为（所有）脂类化合物而已，并不是平行于代谢组学之外的又一新的组学概念。还需要指出的是，本书在第11章中出现了代谢指纹（metabolic fingerprinting）分析一词。这一概念在应用到多细胞生物体的体液成分分析时与本书中的其他概念往往可不加区别。但是，如果应用到单细胞生物或培养细胞体系时就会出现一个与之对应的另一概念——代谢足迹（metabolic footprint）分析。这时候，代谢指纹分析指的是对细胞内（所有）代谢物的定性定量分析，而代谢足迹分析则指的是对分泌到细胞外的（所有）代谢物组分的检测，两者有着明显的分析对象空间限定性。

随着代谢组学概念的日益普及和现代分析技术的发展，国内外对代谢组学的研究也如火如荼地展开。就系统生物学研究而言，没有其他任何一种组学研究可使用如此众多的分析手段，这既为代谢组学的发展提供了广阔空间，同时也为代谢组学进步带来诸多挑战。有关代谢组学的专著国外已经推出许多（我们在2008年出版了国内首部代谢组学专著《代谢组学：原理和应用》），内容各有侧重。由于本书是隶属于《分子生物学方法》系列，在编撰过程中更注重指导性和可操作性，因此，相关分析技术的原理未予过多篇幅和言辞。各章节着重强调实验步骤和流程，相应的数据分析部分更多地是以发现和寻找差异化合物为目的，没有过多地关注数据的生物信息学处理。事实上，随着仪器分析能力和计算机数据储存和处理能力的提高以及各种代谢数据库的建立，数据的生物信息学处理在解决复杂生物学问题中越发显得重要。许多分析仪器生产商已经注意到这一点，集普通统计分析和生物信息学分析功能于一体的商品化的数据处理软件，现在已经可以直接提供到用户手中（比如Agilent公司已经有可以同时整合基因组、转录组、蛋白质组和代谢组数据的商用分析软件推出），这些系统化的软件体系的不断完善也表明，技术层面上，代谢组学的软硬件在不断走向成熟。

事实上，代谢组学（包括本书中所指的代谢轮廓分析）的应用不仅仅体现在先天性代谢缺陷诊断和药物代谢物分析中，生命科学研究的各个领域都在广泛采用代谢组学技术，例如疾病的分型、标志物发现、药物疗效和毒副作用评价、转基因作物实质等同性评估、环境毒理学、遗传学、功能基因组学、微生物生理学及工业发酵等领域都在广泛使用代谢组学策略。本书的潜在读者群十分广泛，既适合业内人士也适合初学者使用。专业的代谢组学研究机构或分析实验室也可以本书为蓝本，参照建立相应标准化分析方案（Standard Operation Protocols）或开展相关工作，教育机构也可以本书作为或编撰实验教材。

中国科学院大连化学物理研究所 代谢组学研究中心
许国旺　高鹏

前　言

在接受为《分子生物学方法》系列丛书编写一卷有关代谢轮廓分析的任务后，我开始揣摩它的涵义所在。Fiehn 将其定义为“对某一完整代谢途径或其交叉通路中特定数目代谢物的定性和定量分析”[1]。与之密切相关的概念还包括代谢靶标分析、代谢指纹分析和代谢组学分析，其中代谢组学分析是指对某一生物系统内代谢物波动的定量检测[2]。上述四种概念常常相互通用。回顾近 40 年的文献资料显而易见，涉及代谢物分析的各概念之间由于分析方法和技术的进步而得以相互关联。譬如，代谢组学的出现不过 10 年而已，但其赖以存在的诸多流程和仪器基础都源于为先天性代谢缺陷诊断和药物代谢分析而建立的各种策略。因此编撰本书时，我试着囊括能直观地展示从单一化合物分子分析向全面的代谢组学分析发展的各种策略。鉴于此，本书将立足于概括性的描述而不是面面俱到，但是我仍希望本书所述方法能够成为代谢轮廓分析领域内的新老同仁的有益资源。

Thomas O. Metz

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（高鹏　译）

Preface

After accepting the task to edit a volume of *Methods in Molecular Biology* devoted to metabolic profiling, I began to contemplate the definition of the term. Fiehn referred to "metabolic profiling" as the identification and quantification of a select number of metabolites in an entire metabolic pathway or intersecting pathways (1). Closely related disciplines were targeted metabolite analysis, metabolic fingerprinting, and metabolomics, the latter of which was defined as the quantitative measurement of perturbations in the metabolite complement of a biological system (2). These four terms are often used interchangeably; indeed, in reviewing the literature over the past 40 years, it is evident that these various disciplines of metabolite analysis are related via an evolution of methods and technology. For example, while the field of metabolomics is now 10 years old, the protocols and instrumentation that form the foundation for the myriad approaches of this discipline are based on those originally established for the diagnosis of inborn errors of metabolism and drug metabolite analysis. Thus, in compiling this volume, I have made an attempt to incorporate protocols that are illustrative of the evolution of metabolic profiling from single molecule analysis to global metabolome profiling. The constraints of this volume necessitate that its contents will be perspective based, rather than comprehensive. However, it is my hope that the methods contained herein will be a resource for both established and new investigators in the field of metabolic profiling.

Thomas O. Metz

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目　录

（高鹏　译）

Contents

Chapter 1

Origins of Metabolic Profiling

Arthur B. Robinson and Noah E. Robinson

Abstract

Quantitative metabolic profiling originated as a 10-year project carried out between 1968 and 1978 in California. It was hypothesized and then demonstrated that quantitative analysis of a large number of metabolites – selected by analytical convenience and evaluated by computerized pattern recognition – could serve as a useful method for the quantitative measurement of human health. Using chromatographic and mass spectrometric methods to measure between 50 and 200 metabolites in more than 15,000 human specimens, statistically significant and diagnostically useful profiles for several human diseases and for other systematic variables including age, diet, fasting, sex, and other variables were demonstrated. It was also shown that genetically distinct metabolic profiles for each individual are present in both newborn infants and adults. In the course of this work, the many practical and conceptual problems involved in sampling, analysis, evaluation of results, and medical use of quantitative metabolic profiling were considered and, for the most part, solved. This article is an account of that research project.

Key words: Metabolic profiling, metabolomics, urine, breath, chromatography, mass spectrometry, aging, diagnostic medicine, preventive medicine.

1. Introduction

Since the dawn of the age of modern chemistry, biochemistry has been of great interest. When molecular structure became established as an exact discipline, the minds of scientists naturally turned toward those molecules of which they themselves are made. Extensive cataloging and structure determination of these substances followed.

As the role of proteins in catalyzing the chemical reactions of metabolism was revealed, progress was made in understanding the metabolites – the smaller molecules required for life that protein

T.O. Metz (ed.), *Metabolic Profiling*, Methods in Molecular Biology 708,
DOI 10.1007/978-1-61737-985-7_1,

catalysts select from the many atomic combinations available and produce to make life possible.

Detailed understanding of metabolism was not, however, possible until the discovery of carbon 14 (1) and the development of tracer methodology (2), which now includes both radioactive and stable isotopes. When it became possible to label the atoms of metabolites and trace their paths through living systems, a thorough understanding of metabolism was achievable.

This understanding and the rapid advance of protein chemistry then led to explanations for some of the simplest metabolic diseases – genetic errors that cause well-defined inborn errors of metabolism. As analytical technology advanced, the list of known genetic illnesses expanded to include a large number of such diseases which, while individually rare, together cause much suffering. This work was further accelerated by findings that, in some cases such as phenylketonuria, understanding of the disease could lead to effective therapy.

Simultaneously, improvements in analytical chemistry led to a search for single metabolites that are diagnostic of more prevalent diseases – including those with non-genetic components. An extensive armament of single-substance measurements entered the inventory of clinical laboratories – tests for both inborn errors and other illnesses. Businesses arose to measure these substances, primarily in blood and urine, which have now grown in the United States alone into a $100 billion industry.

This work usually involved the correlation of one substance with a condition of interest in human health. Scientists searched for metabolites and proteins, the quantities of which contained sufficient information about health and disease to warrant their measurement. A few such measurements became standard in health screening of ordinary patients, while a much larger number were made available in clinical laboratories, available upon request by physicians for specific patients.

While the many substances measureable in human samples were increasingly evident as analytical methods improved, no practical efforts were made to test the possibility that the simultaneous quantitative analysis of large numbers of metabolites followed by computerized pattern recognition could yield health information of significant value.

Forty years ago, however, there arose in California an experimental project with the potential to cause a paradigm shift toward the use of simultaneous measurement of large numbers of metabolites for the quantitative measurement of human health. This effort was ahead of its time and, therefore, faced daunting challenges in the construction of analytical and computational capabilities.

This work was known in the 1970s as "quantitative metabolic profiling." It is now a growing part of "metabolomics." While

metabolomics still contains substantial single-substance components, extraordinary advances in analytical and computational technology are rapidly moving this field toward metabolic profiling – a continuation of that 1970s' effort with greatly superior modern analytical equipment and computers.

The California work was funded by private donors, NIH grants, and the personal savings of some of the scientists themselves. This effort proved the enormous analytical power of metabolic profiling as applied to human tissues and developed new analytical and computational tools. It had its origin in a collaboration beginning in 1968 between Linus Pauling and Art Robinson at the University of California at San Diego (UCSD). Later, it continued at Stanford University and the Institute of Orthomolecular Medicine (later renamed the Linus Pauling Institute of Science and Medicine), which Pauling and Robinson cofounded in Menlo Park, California, in 1973.

2. Orthomolecular Psychiatry

Pauling hypothesized (3) that the distribution functions of optimum human nutritional requirements are very wide, leading to nutritional deficiencies and illness, especially mental illness, in many people. He invented the term "orthomolecular psychiatry" – meaning right molecule in the right amount for mental health – to designate the treatment of mental illness by means of megavitamin therapy. Later this was designated "orthomolecular medicine" to include treatment of other illnesses in a similar way.

Having worked together at Caltech in 1962–1963 on the chemical basis of general anesthesia (4), both Pauling and Robinson were faculty members at UCSD when Pauling made this proposal. At the time, Pauling was developing a theory of the structure of the atomic nucleus, and Robinson and his students were studying the deamidation of asparginyl and glutaminyl residues in peptides and proteins. In addition to this ongoing work, in 1968 the two men began a collaboration to test Pauling's ideas about orthomolecular psychiatry, with Robinson directing the experimental work and Pauling extending the theoretical aspects, which led eventually to his widely known hypotheses concerning the role of vitamin C in health and disease.

Pauling initially proposed an experimental program using vitamin-loading tests, in which large doses of vitamins were given to experimental subjects – those having mental illnesses and control subjects – and the urinary excretion of the vitamins measured. It was postulated that those individuals with greater needs for the substances would retain more, excreting lesser amounts in

their urine. Robinson assembled a small research group and set up a laboratory for this purpose, while continuing to direct his own laboratory – the size of which was increased by UCSD to accommodate the new work. The initial experiments emphasized loading tests with ascorbic acid, niacin, and pyridoxine, and some interesting results were obtained.

It soon became evident, however, that this approach was of less value than hoped. The experiments gave very limited information, and the necessary analytical procedures of that day were laborious, time consuming, and expensive, which diminished their practical value.

3. Origin of the Profiling Hypothesis

In the course of this work, Robinson utilized a method for measurement of pyridoxine in chemically derivatized urine by means of packed-column gas chromatography, which involved resolution of the pyridoxine peak from the large number of metabolic products that are present in urine. During these experiments, Robinson began to think that the information they needed might be more readily available in the many metabolic constituents evident in the chromatograms rather than in the pyridoxine peak itself. He reasoned as follows. The fundamental need was for a method to measure health vs. the amounts of ingested nutrients, as is illustrated in **Fig. 1.1**. This required, however, a means of measuring metabolic health quantitatively. He hypothesized that the needed values might be obtained by measuring the amounts of a large sampling of urinary metabolites and statistically correlating the patterns in these profiles with various states of human health and disease. Robinson, therefore, initiated an experimental program, with Pauling's support, to test the hypothesis that quantitative metabolic profiles contained sufficient information for this purpose. As this work progressed, he assembled a skilled group of co-workers for this project.

4. Scientists Who Tested the Hypothesis

These included Roy Teranishi, Dick Mon, and Robert Flath – highly skilled experts in gas chromatography; Martin Turner and Carl Boehme – engineers who built and maintained the PDP-11 vintage computer hardware used for lab automation and data collection; Laurelee Robinson – who wrote the computer software