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Special issue

Dynamic Metabolic Networks

Editors: Nick Kruger and George Ratcliffe

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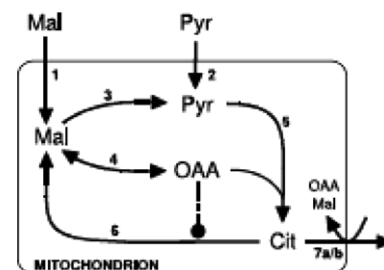
Preface	pp 2134–2135
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NETWORK PROPERTIES

Computational approaches to the topology, stability and dynamics of metabolic networks	pp 2139–2151
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Ralf Steuer

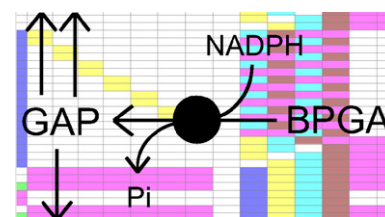
Recent computational strategies to evaluate the dynamic capabilities of metabolic networks are described and exemplified using paradigmatic models of metabolic pathways.



Measuring <i>in vivo</i> elasticities of Calvin cycle enzymes: Network structure and patterns of modulations	pp 2152–2162
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Michael Kreim, Christoph Giersch*

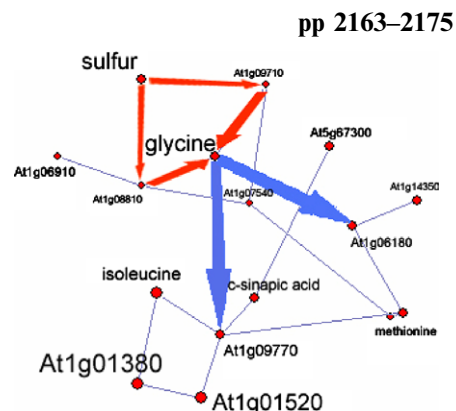
Enzyme kinetics can be studied under *in vivo* conditions by modulating the activities of certain target enzymes. We extend this approach and apply it to the Calvin photosynthesis cycle with the aim to identify the set of modulations that allows maximum information gain at minimal experimental effort.



On the processing of metabolic information through metabolite–gene communication networks: An approach for modelling causality

Jedrzej Szymanski, Monika Bielecka, Fernando Carrari, Alisdair R. Fernie, Rainer Hoefgen, Victoria J. Nikiforova*

The dynamic behaviour of biological systems is accomplished through informational exchange at different levels of the cellular hierarchy. Here, we present an approach integrating metabolic and transcript data into a causally directed network of inter-level informational flows. Using simple statistical tools we identify putative metabolic regulators of the adaptive response to environmental and developmental challenges.



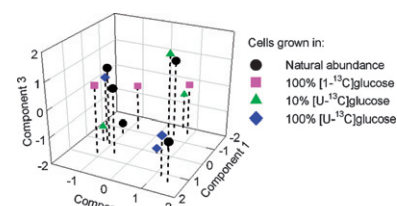
STEADY-STATE FLUX ANALYSIS

Network flux analysis: Impact of ^{13}C -substrates on metabolism in *Arabidopsis thaliana* cell suspension cultures

pp 2176–2188

Nicholas J. Kruger*, Joanna E. Huddleston, Pascaline Le Lay, Nicholas D. Brown, R. George Ratcliffe*

Cell suspension cultures of *Arabidopsis thaliana* grown in media differing in ^{13}C -enrichment are metabolically indistinguishable. There was no significant difference in the pattern of metabolism of either specific ^{14}C -labelled or $[\text{U-}^{14}\text{C}]$ glucose between the cultures. Principal component analysis of ^{13}C -decoupled ^1H NMR metabolite fingerprints of cell extracts failed to discriminate between the different culture conditions. It is concluded that ^{13}C -enrichment of the growth substrate has no effect on flux through the network of central carbon metabolism.

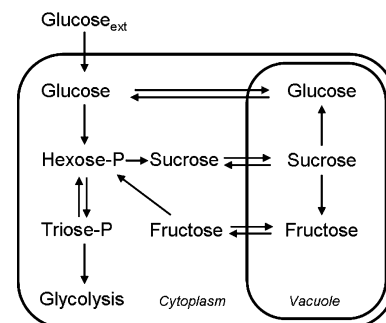


Vacuolar compartmentation complicates the steady-state analysis of glucose metabolism and forces reappraisal of sucrose cycling in plants

pp 2189–2196

Nicholas J. Kruger*, Pascaline Le Lay, R. George Ratcliffe*

Failure to consider vacuolar compartmentation of glucose can have a marked influence on estimates of sucrose cycling from steady-state metabolic flux analysis. Mathematical modelling shows that measurements of the labelling of both cytosolic and vacuolar glucose are required to resolve the fluxes involving intracellular glucose in plants.

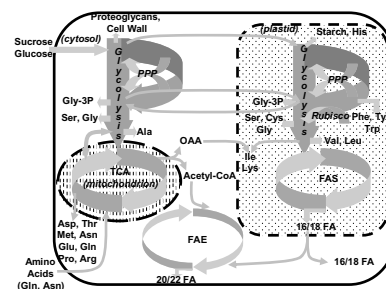


Compartment-specific labeling information in ^{13}C metabolic flux analysis of plants

pp 2197–2210

Doug K. Allen*, Yair Shachar-Hill, John B. Ohlrogge

Plants and particularly their seeds have great potential for low cost production of chemical feedstocks and novel compounds, but rational metabolic engineering requires a better understanding of central carbon metabolism. To aid flux quantification we report new and improved measurements of ^{13}C labeling information from specific compartments within the plant cell.

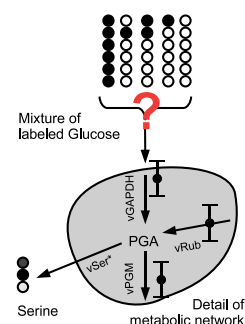


Design of substrate label for steady state flux measurements in plant systems using the metabolic network of *Brassica napus* embryos

Igor G.L. Libourel*, Jackson P. Gehan, Yair Shachar-Hill

Rapeseed embryos are a model system for studying plant metabolism. In steady state ^{13}C -feeding experiments, metabolic fluxes are determined using measurements of labeling in metabolic end-products. Using previously measured flux values, this criterion-based optimal design study determines substrate label combinations that provide the highest possible information content.

pp 2211–2221

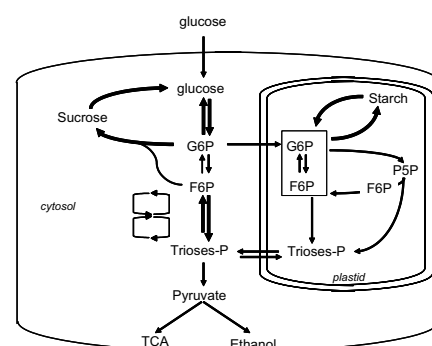


Substrate cycles in the central metabolism of maize root tips under hypoxia

Ana Paula Alonso, Philippe Raymond, Dominique Rolin, Martine Dieuaide-Noubhani*

This work describes a metabolic flux analysis (C13 and C14 labeling experiments for flux quantification) performed to study the response of maize root tips to hypoxia. ATP production was severely reduced, and the flux through the substrate cycles all decreased. However, substrate cycles remain important in terms of ATP consumption (50% of the produced ATP) and their importance was discussed.

pp 2222–2231

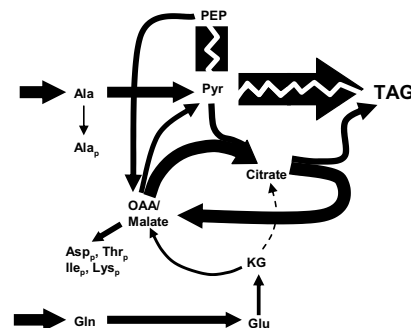


Parallel determination of enzyme activities and *in vivo* fluxes in *Brassica napus* embryos grown on organic or inorganic nitrogen source

Björn H. Junker, Joachim Lonien, Lindsey E. Heady, Alistair Rogers, Jörg Schwender*

Brassica napus embryos were grown in culture with either glutamine and alanine or ammonium nitrate as the sole nitrogen source. Dependent on the nitrogen source, *in vivo* metabolic fluxes around the TCA cycle changed distinctly. The changes observed in enzyme activity were not consistent with the changes in metabolic flux. It is suggested that the observed flux adjustments are driven by mass balances rather than transcriptional regulation.

pp 2232–2242

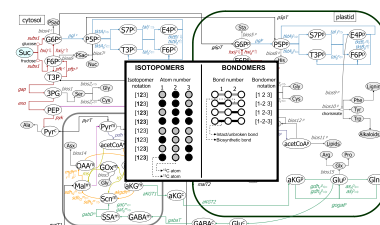


Flux quantification in central carbon metabolism of *Catharanthus roseus* hairy roots by ^{13}C labeling and comprehensive bondomer balancing

Ganesh Sriram, D. Bruce Fulton, Jacqueline V. Shanks*

Metabolic flux quantification is a powerful profiling tool in plant metabolic engineering and systems biology. We introduce the application of “bondomers”, a computationally efficient and intuitively appealing alternative to the commonly used “isotopomers”, toward systemic evaluation of fluxes in central carbon metabolism of *Catharanthus roseus* hairy roots.

pp 2243–2257



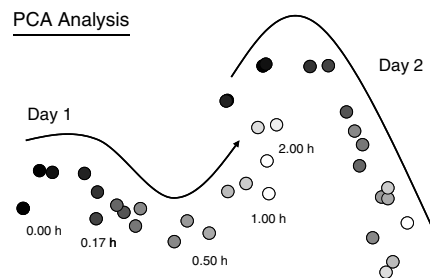
DYNAMIC FLUX ANALYSIS

GC-EI-TOF-MS analysis of *in vivo* carbon-partitioning into soluble metabolite pools of higher plants by monitoring isotope dilution after $^{13}\text{CO}_2$ labelling

pp 2258–2272

Jan Huege, Ronan Sulpice, Yves Gibon, Jan Lisec, Karin Koehl, Joachim Kopka*

The monitoring of isotope dilution after $^{13}\text{CO}_2$ labelling was optimized using *Arabidopsis thaliana* Col-0 or *Oryza sativa* IR57111 plants, which were maximally labelled with ^{13}C . Carbon isotope dilution was evaluated for short (2 h) and long-term (3 days) kinetic measurements of metabolite pools in roots and shoots. Both approaches were shown to enable the characterization of metabolite specific partitioning processes and kinetics. A current experimental design for the kinetic metabolic phenotyping of higher plants using GC-EI-TOF-MS profiling analysis is proposed.

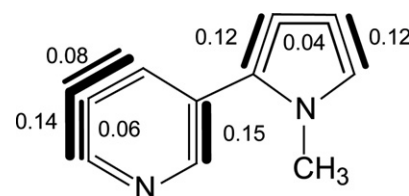


$^{13}\text{CO}_2$ as a universal metabolic tracer in isotopologue perturbation experiments

pp 2273–2289

Werner Römisch-Margl, Nicholas Schramek, Tanja Radykewicz, Christian Ettenhuber, Eva Eylert, Claudia Huber, Lilla Römisch-Margl, Christine Schwarz, Maria Dobner, Norbert Demmel, Bernhard Winzenhörlein, Adelbert Bacher, Wolfgang Eisenreich*

The pilot study shows that pulse/chase labeling with $^{13}\text{CO}_2$ as precursor is a powerful tool for the study of quantitative aspects of plant metabolism in completely unperturbed whole plants.

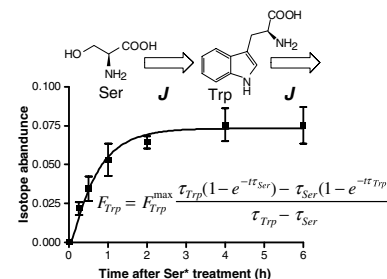


Metabolic flux analysis in plants using dynamic labeling technique: Application to tryptophan biosynthesis in cultured rice cells

pp 2290–2301

Fumio Matsuda, Kyo Wakasa, Hisashi Miyagawa*

The concept and methodology of dynamic labeling for metabolic flux analysis (MFA) of plant metabolic pathways are discussed by describing a MFA study of tryptophan biosynthetic pathway in cultured rice cells.

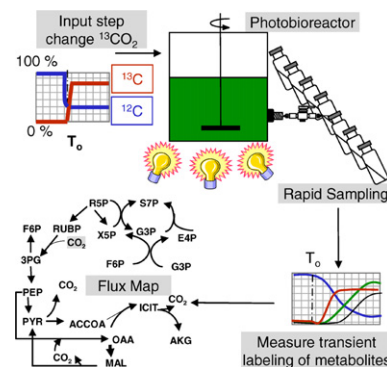


A transient isotopic labeling methodology for ^{13}C metabolic flux analysis of photoautotrophic microorganisms

pp 2302–2312

Avantika A. Shastri, John A. Morgan*

A transient ^{13}C metabolic flux analysis methodology to measure central carbon fluxes in purely photoautotrophic systems under metabolic steady-state is formulated. A mathematical framework of ^{13}C isotopomer balances is used to assess various experimental requirements of the problem, including intracellular metabolite concentration measurements and photobioreactor operation.

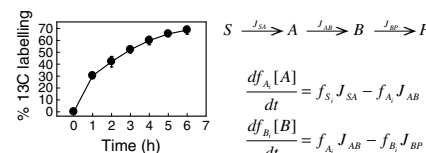


Determination of metabolic fluxes in a non-steady-state system

pp 2313–2319

C.J. Baxter, J.L. Liu, A.R. Fernie, L.J. Sweetlove*

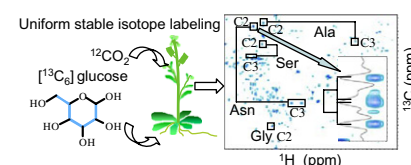
At non-steady-state, the labelling of a metabolite pool can be described by equations based on mass-balance of labelled molecules. Non-steady-state fluxes can be estimated from such equations given data on change in labelling and metabolite pool size.

**METHODOLOGY****Towards dynamic metabolic network measurements by multi-dimensional NMR-based fluxomics**

pp 2320–2329

Yasuyo Sekiyama, Jun Kikuchi*

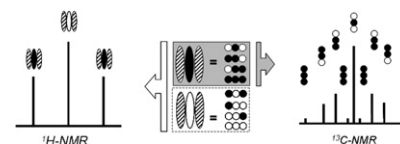
We propose an approach for measuring dynamic metabolic networks that combines different extraction solvents and NMR pulse sequences. In addition, we present examples that involve monitoring the time-dependent changes in the ^{13}C -bondomer composition of *Arabidopsis thaliana* labeled with $^{13}\text{C}_6$ glucose.

**NMR-based fluxomics: Quantitative 2D NMR methods for isotopomers analysis**

pp 2330–2340

Stéphane Massou, Cécile Nicolas, Fabien Letisse, Jean-Charles Portais*

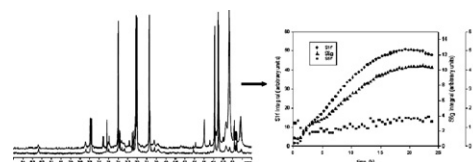
Combined with ^{13}C -labelling experiments, NMR provides two different types of data valuable for the analysis of metabolic fluxes: specific enrichments and positional isotopomers. Both data types can be measured for various metabolites in complex mixtures by using relevant quantitative 2D-NMR methods.

**In vivo ^{13}C NMR determines metabolic fluxes and steady state in linseed embryos**

pp 2341–2350

Stéphanie Troufflard, Albrecht Roscher*, Brigitte Thomasset, Jean-Noël Barbotin, Stephen Rawsthorne, Jean-Charles Portais

The analysis of time-course data from ^{13}C -labelling experiments detected by *in vivo* NMR of developing linseed embryos is shown to be complementary to steady state metabolic flux analysis as it gives information on the isotopic and metabolic dynamics while reaching steady state and allows determination of complementary fluxes.

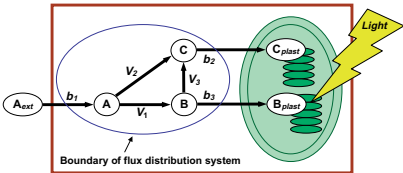


Experimental and mathematical approaches to modeling plant metabolic networks

pp 2351–2374

Rigoberto Rios-Esteva, Bernd Markus Lange*

This article provides an overview of approaches for evaluating the control of metabolic flux in plants, with an emphasis on using simplified case studies and examples from the plant metabolism literature, to demonstrate the utility of combining mathematical modeling with experimental testing. The mathematical concepts are explained and discussed with a target readership of phytochemists, biochemists, biophysicists and geneticists in mind.



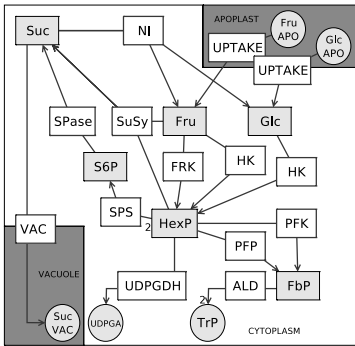
KINETIC MODELLING

Kinetic model of sucrose accumulation in maturing sugarcane culm tissue

pp 2375–2392

Lafras Uys, Frederik C. Botha, Jan-Hendrik S. Hofmeyr, Johann M. Rohwer*

Kinetic modelling was used to investigate sucrose accumulation in the storage parenchyma of sugarcane (*Saccharum officinarum*). This approach yielded a profile of metabolic changes associated with the maturation of sugarcane internodes. The control over the sucrose accumulation process could also be quantified.

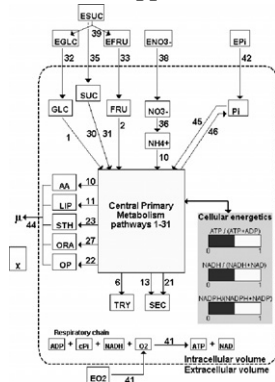


Dynamic flux cartography of hairy roots primary metabolism

pp 2393–2404

M. Cloutier, M. Perrier, M. Jolicoeur*

A dynamic metabolic model is proposed to analyze and visualize hairy roots primary metabolism. The visualization allows for a better understanding of the interactions between nutrients, metabolites and pathways in plant metabolism.



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