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Editorial

# Dynamic metabolic networks: Going with the flow

The apparently mundane activity of identifying and quantifying metabolites has seen a huge increase in activity in recent years. In the process it has been transformed into a discipline in its own right, metabolomics, and this has extended beyond the technology-driven creation of large datasets to embrace the development of novel ways of using metabolic data. A snap shot of plant metabolomics was published in an earlier Special Issue of *Phytochemistry* in 2003, and the rapid growth of the field since then has more than justified the optimism expressed by the editors at that time (Dixon and Strack, 2003).

This upsurge in metabolic research is especially welcome, because our understanding of the control and regulation of compartmented plant metabolic networks has not yet translated into the truly predictive models of central metabolism that would permit rational metabolic engineering (Kruger and Ratcliffe, in press). Metabolic networks are dynamic entities, supporting the flow of material that underpins biological activity, and extending the focus of metabolomics to include measurements of flux is a logical development (Fernie et al., 2005). Thus, as argued elsewhere (Ratcliffe and Shachar-Hill, 2005), metabolic phenotypes based on flux measurements complement those based on metabolite composition, and this extra dimension is now reflected in the routine inclusion of flux sessions at metabolomics meetings.

Flux analysis, like metabolite analysis, has a long history in experimental plant science (James, 1953; ap Rees and Hill, 1994), and its recent re-emergence as a mainstream activity has been documented in several review articles (Kruger et al., 2003; Schwender et al., 2004; Ratcliffe and Shachar-Hill, 2006; Wiechert et al., 2007). The development of the field has been propelled by continuing improvements in analytical methods and by the development of increasingly powerful tools for analysing the data. A key feature of current research is the attempt to produce comprehensive system-wide flux maps, and inevitably the term fluxomics has crept into the literature as a convenient shorthand for flux analysis. This rebranding carries the risk of suggesting the existence of robust, easily applicable, high-throughput methods; whereas the reality, at least for plants, is that flux analysis is a more complicated activity than the detection and quantitation of particular classes of macromolecule or suites of metabolites.

There are several reasons why flux analysis is a challenging activity. First, there is no single agreed strategy for determining fluxes, and in practice it is usually necessary to assemble the components of a flux map from a series of different measurements. Secondly, there is often no simple correspondence between the measurements and the deduced fluxes. Thus while an input or output flux can usually be determined relatively easily, the fluxes that relate inputs and outputs are more elusive and only emerge as the result of a computational exercise based on a model of the metabolic network. Finally, the output of a flux analysis is not a direct measurement of quantity, but a first derivative with respect to time, and this inevitably requires a more stringent analytical and statistical procedure to determine the output accurately. Fortunately the obstacles are not insuperable, and flux analysis in bacteria at least has developed to the point where the term fluxomics is appropriate (Sauer, 2004; Fischer and Sauer, 2005).

In the belief that flux analysis has an equally important role to play in analysing the processes that underpin the growth and productivity of plants, this Special Issue in Dynamic Metabolic Networks brings together a collection of articles on the theory and practice of network flux analysis in plants. The field is of necessity interdisciplinary, and while much of the Special Issue is devoted to progress in flux measurement – hence the uncompromising message of the front cover illustration – it is important to have an appreciation of the inherent properties of the networks that support the fluxes. With this in mind the issue includes articles by Steuer on a new way of modelling the dynamic properties of metabolic networks, by Kreim and Giersch proposing an efficient general method for determining the elasticities of all the enzymes in a network, and by Nikiforova et al. exploring the correlations that can be observed between transcript and metabolite levels. The concepts that underpin these papers may seem remote from the day-to-day research activity of many readers, but they need to be grasped and taken further in the hope that a more

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sophisticated description of the metabolic network will eventually lead to a complete understanding of the complex metabolic phenotypes exhibited by plants.

However in the words of the English novelist and poet D.H. Lawrence, ''Only the flow matters.'' (Boulton, 2004) and with this in mind most of the remaining articles focus on flux measurement. Two general methods are available – steady-state and dynamic (kinetic) analysis – and although both depend on labelling experiments, they differ greatly in almost every other respect (Ratcliffe and Shachar-Hill, 2006).

Steady-state analysis depends on measuring the redistribution of a label after a system has attained an isotopic and metabolic steady-state. When combined with a knowledge of the input and output fluxes for the system, this information is sufficient to generate a flux map that defines the forward and reverse fluxes between branch points in the metabolic network. Steady-state labelling is particularly suitable for analysing the flux distribution through the central pathways of carbon metabolism. It generates a descriptive flux map of the network, and while the necessity for an isotopic and metabolic steady-state is restrictive, the experience with microbial systems has demonstrated that the results of steady-state flux analysis can be a powerful tool for metabolic engineering.

Steady-state analysis is well represented in the Special Issue and the contributions fall into two categories: papers exploring the assumptions of the method and the way in which it is used; and papers reporting flux maps for plants systems under particular physiological conditions. In the first category, Kruger et al. show first, that the non-tracer amounts of label used in stable isotope steady-state analysis cause no detectable perturbation of the metabolic network, and secondly, that information on the subcellular origin and compartmentation of labelled metabolites is essential for a correct analysis of the flux distribution in central metabolism. Allen et al. then describe a range of improved techniques for obtaining compartment-specific labeling information; and Libourel et al. discuss the criteria for choosing the best combination of substrates for a steady-state labelling experiment. In the second category: Alonso et al. discuss the impact of hypoxia on the flux map for maize root tips; Junker et al. discuss the effect of different nitrogen sources on the flux map for cultured *Brassica napus* embryos; and Sriram et al. provide a detailed flux map for the central metabolism in a hairy root culture of *Catharanthus roseus*. Overall these papers suggest that steady-state flux analysis in plants has a firm foundation, and that it has progressed to the point where it can generate novel information about the performance of particular metabolic networks.

In contrast to steady-state analysis, dynamic analysis depends on measuring the kinetics of label redistribution and pool size during labelling time-courses. It is more demanding than steady-state analysis, both experimentally and computationally, and it suffers from inherent limitations in complex networks. On the other hand dynamic labelling avoids the restrictive assumptions of the steady-state method, and it has the potential to generate predictive models of the flux distribution. The dynamic labelling method for flux measurement is less well developed than the steady-state method, but there have been some notable applications in the analysis of secondary plant metabolism and there is now a keen interest in finding a way to extend the method to central carbon metabolism.

Reflecting the state of the field, the dynamic labelling papers in the Special Issue differ strikingly in scope and applicability. Huege et al. generate fully labelled plants by growth in an atmosphere containing $^{13}CO_2$, and then monitor the half-time for dilution of the label in specific pools following the return to ambient $CO_2$. In contrast Eisenreich et al. expose their plants to a short pulse of $^{13}CO_2$ and then follow the redistribution of the label in a subsequent chase period. Both approaches can be analysed quantitatively and have phenotypic value for comparative analysis. In yet another approach several authors analyse the time-courses during continuous labelling: Matsuda et al. provide an illustrative example of the application of the method to tryptophan biosynthesis; Shastri and Morgan discuss their progress in extending the method to photoautotrophic metabolism in *Synechocystis*; and Baxter et al. describe a method for deriving fluxes from mass isotopomer data for metabolites extracted from a heterotrophic *Arabidopsis thaliana* cell culture. Taken together these papers exemplify the exploratory nature of the field, with an emphasis on establishing robust protocols in diverse plant systems.

Both steady-state and dynamic flux analysis are very dependent on the availability of analytical methods and computational tools for measuring the redistribution of isotopic labels. The pre-eminent analytical techniques are nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS) (Ratcliffe and Shachar-Hill, 2006), and while many of the papers in the Special Issue discuss methodological aspects of NMR and MS in relation to particular applications, three papers focus exclusively on developments in NMR that should aid flux analysis. Thus Kikuchi et al. show how two dimensional (2D) NMR methods can simplify the isotopomeric analysis of metabolites extracted from plants grown on a mixture of uniformly labelled and unlabelled substrates; Massou et al. describe the optimisation of the 2D-TOCSY experiment to provide a powerful method for measuring positional enrichment; and Troufflard et al. provide a timely reminder of the non-invasive nature of NMR, by using *in vivo* NMR to monitor the evolution of the isotopic and metabolic steady-state in abundant metabolites in linseed embryos. Full value can only be obtained from these increasingly sophisticated techniques if the computational tools are available to interpret the data. Experimentalists often have to forge collaborations to gain access to the relevant expertise and the review by Rios-Estepa and Lange provides a useful description of some of the principal computational tools that are encountered in flux analysis and modelling.

The alternative to measuring fluxes is to predict them, and two papers discuss the construction of kinetic models

that allow such predictions. Uys et al. extend an existing model on sucrose accumulation in sugar cane and use it to analyses the futile cycling that occurs between synthesis and breakdown; while Cloutier et al. continue the development of a large-scale model for primary metabolism in hairy root cultures of *Catharanthus roseus*. The development of kinetic models depends critically on the availability of kinetic constants for the enzymes, and if robust methods can be established for analysing dynamic labelling experiments then it should be possible to use the mechanistic models that emerge from that analysis as a template for kinetic modelling *in silico*.

In conclusion the aim of the Special Issue is to stimulate interest in the dynamic properties of plant metabolic networks and to accelerate the development of robust methods for the quantitative analysis of the fluxes they support. In due course this will lead to the development of mechanistic models of plant metabolism that will encapsulate a complete description of the control and regulation of metabolic networks under the full range of relevant physiological conditions; and simultaneously these models will be fully predictive and thus the ideal tool for efficient and effective metabolic engineering. Happily there is much to be done in the mean time, and it is hoped that readers of the Special Issue will return to the bench or the computer screen with renewed enthusiasm for the task ahead.

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## References

ap Rees T., Hill S.A., 1994. Metabolic control analysis of plant metabolism. Plant Cell Environ. 17, 587–599.

Boulton J.T., 2004. D.H. Lawrence: Late Essays and Articles. Cambridge University Press, pp. 149–154.

Dixon R.A., Strack D., 2003. Phytochemistry meets genome analysis, and beyond …….. Phytochemistry 62, 815–816.

Fernie A.R., Geigenberger P., Stitt M., 2005. Flux an important, but neglected, component of functional genomics. Curr. Opin. Plant Biol. 8, 174–182.

Fischer E., Sauer U., 2005. Large-scale in vivo flux analysis shows rigidity and suboptimal performance of *Bacillus subtilis* metabolism. Nat. Genetics 37, 636–640.

James W.O., 1953. Plant Respiration. Clarendon Press, Oxford.

Kruger, N.J., Ratcliffe, R.G., in press. Metabolic organization in plants: a challenge for the metabolic engineer. In: Bohnert, H.J., Nguyen, H.T. (Eds.), Advances in Plant Biochemistry and Molecular Biology, Bioengineering and Molecular Biology of Plant Pathways, vol. 1, Elsevier.

Kruger N.J., Ratcliffe R.G., Roscher A., 2003. Quantitative approaches for analyzing fluxes through plant metabolic networks using NMR and stable isotope labeling. Phytochem. Rev. 2, 17–30.

Ratcliffe R.G., Shachar-Hill Y., 2005. Revealing metabolic phenotypes in plants: inputs from NMR analysis. Biol. Rev. 80, 27–43.

Ratcliffe R.G., Shachar-Hill Y., 2006. Measuring multiple fluxes through plant metabolic networks. Plant J. 45, 490–511.

Sauer U., 2004. High-throughput phenomics: experimental methods for mapping fluxomes. Curr. Opin. Biotechnol. 15, 58–63.

Schwender J., Ohlrogge J., Shachar-Hill Y., 2004. Understanding flux in plant metabolic networks. Curr. Opin. Plant Biol. 7, 309–317.

Wiechert W., Schweissgut O., Takanaga H., Frommer W.B., 2007. Fluxomics: mass spectrometry versus quantitative imaging. Curr. Opin. Plant Biol. 10, 323–330.

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