Chasing the flux:

Inferring pathways from the flux analysis of carbon metabolism

Evangelos Simeonidis, Kieran Smallbone, Warwick Dunn, Neil Swainston, Pedro Mendes, Hans V. Westerhoff

Manchester Centre for Integrative Systems Biology, MIB, Manchester, M1 7DN, Great Britain

Introduction

One of the goals of Systems Biology is to develop and utilise high-throughput methods for the measurement of kinetic parameters on a genome-wide scale, whilst at the same time generating predictive models for system behaviour. In studying large-scale networks such as metabolism, the task of exhaustively assaying and measuring all the components of reactions can be costly, and time-consuming. It would mean the assaying and measurement of hundreds or even thousands of enzymes (their activities and their concentrations) for the construction of a full-scale, full-detail model. There is a clear need for strategies that allow us to systematically select the subsets of reactions and pathways which should be prioritized when studying metabolism.

Here, we describe the development of a methodology that can be used to select those reactions that carry the overwhelming majority of carbon flux through the metabolic network of an organism.

Strategy

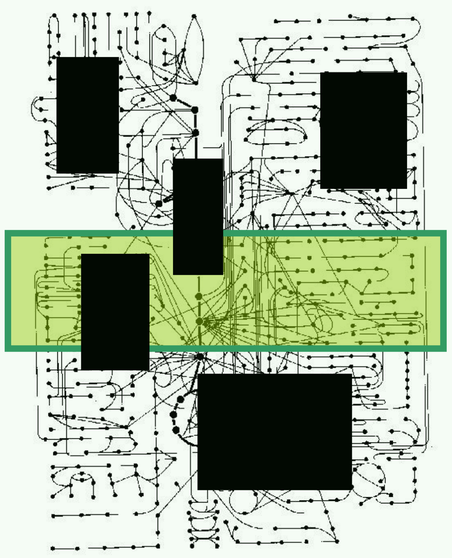

Figure 1 - In kinetically characterizing the proteins of a genome, substrates necessary for the assays are missing, assays are too indirect, or other enzymes interfere. Even missing some of the enzymes at random on the metabolic map means that no complete pathway can be modelled and understood. One might characterise 90% of metabolic enzymes, but only 10% of flux or system function. If we instead focus on a smaller number of enzymes that take part in defined major flux pathways, then we will get closer to understanding full function.

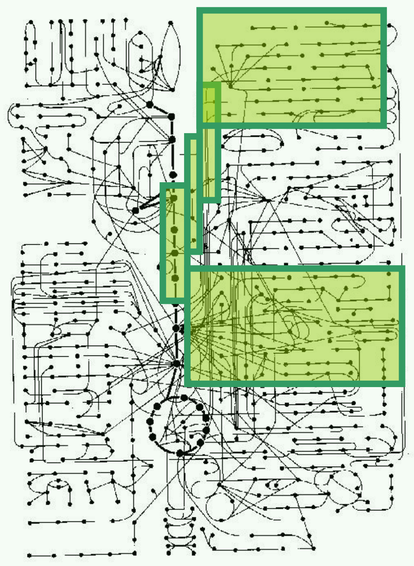

Figure 2 - We derive a new strategy, which focuses on major flux through the network. The first requirement is a metabolic network. The recent community-driven, highly-curated reconstruction [Herrgård *et al.*, *Nat. Biotechnol.* 2008, 26:1155] provides the basis for our analysis. We define relevant experimental conditions where the flux patterns are expected to be fairly simple and predict mathematically (flux balance analysis, FBA) and/or experimentally (metabolic flux measurements) which reactions carry the major fluxes through the the network.

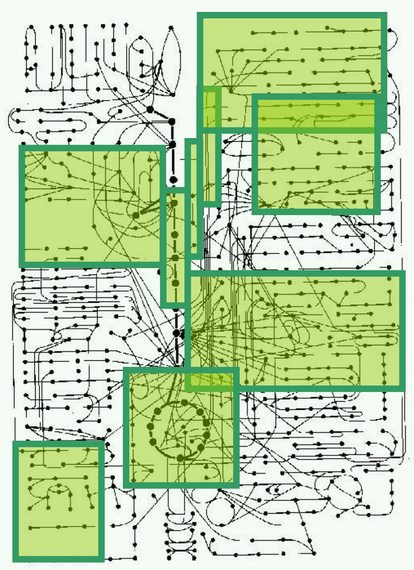

Figure 3 - The enzymes are then ranked according to most flux, and organised in flux-carrying networks with the application of an elementary flux mode (EFM) analysis. Less than 20% of all enzymes carry more than 99% of total flux. We prioritise these for experimental study and continue towards higher and higher flux percentage. These enzymes can be characterized kinetically and a silicon cell type “bottom up” model can be created.

Carbon flux coverage

The analysis shows that as much as 98% of the total carbon flux can be covered by analysing a small number of enzymes.

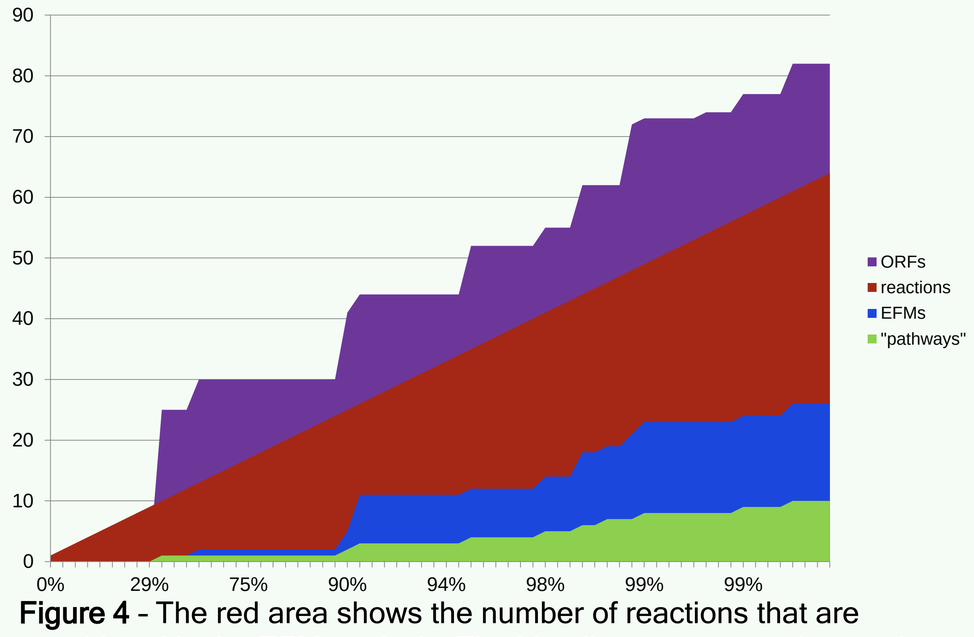

Figure 4 – The red area shows the number of reactions that are considered in the EFM analysis. The blue line represents the number of EFMs produced, and the green line tries to capture the traditional metabolic pathways that the EFMs correspond to. The purple line enumerates the number of enzymes that catalyse the rections in the selected EFMs and that would need to be studied to cover most of the carbon flux.

Conclusion

The new strategy allows an exponentially faster coverage of the flux carrying reactions in the metabolic network of an organism. Experiments can prioritise these reactions and their enzymes, ensuring that major functions of the organism will be covered first.

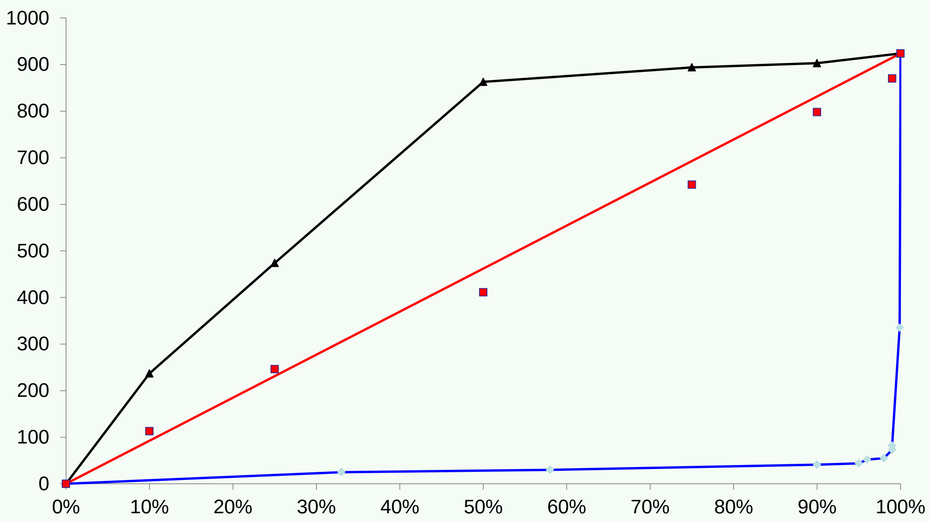

Figure 5 – Our strategy (blue line) approaches a flux coverage of more than 90% with only a small number of enzymes. A random progression (red line) would advance much slower. In reality, progress would be even slower (black line), because of the effects of missing substrates, indirect assays or other difficulties with the experimental characterisation.

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For more information please contact v.simeonidis@manchester.ac.uk