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Kinetic modelling of large-scale metabolic networks

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ABSTRACT

A major result of the various genome programs has been an accumulation of complete genomic sequences and their associated annotation. These resources are extremely valuable to various fields of biology, not least metabolism and metabolic modelling. As these complete sequences have started appearing they have been used to derive lists of metabolic reactions that are catalysed by enzymes whose genes are identified in the genome sequence. These metabolic "reconstructions" are further interpreted as metabolic networks and several analyses can be derived from them. In the case of the popular model organism *Saccharomyces cerevisiae* the metabolic reconstruction is fairly advanced in terms of completeness and sophistication [2, 1].

On its own, a metabolic reconstruction can be analysed through a number of approaches: network analyses (clustering coefficients, betweeness centrality, etc.) provide metrics about the connectedness of the network; elementary flux mode analysis provides a unique decomposition of the network in minimal subsets that are capable of operating independently. By joining extra quantivative information about input and output fluxes, the network can also be studied using flux balance analysis [3]. These methods require little amount of molecular information, however they are only able to provide a restricted number of steady state properties of the system. In order to reveal the network's dynamic properties, kinetic models are required. Here we provide an account of our efforts towards costructing and analysing such large scale kinetic models of metabolism.

Kinetic models describe the dynamic properties of reaction networks and are formulated based on the kinetic properties of the individual reactions/enzymes of the network. Traditionally the kinetics are determined through *in vitro* studies, which require purification of the enzymes involved. Studied in that way, each enzyme can be characterized by a detailed kinetic rate law — each enzymatic reaction with a specific rate law. This is a problem because even for the best studied organisms, such as *S. cerevisiae*, the large majority of enzymes has never been studied, and so the rate laws and their parameter values are unknown. Systematic efforts to purify large numbers of enzymes and determine their precise kinetic properties are under way in our Centre, but this cannot scale all the way to the entire set of enzymes of the network. Nevertheless we have already determined such detailed kinetics for all enzymes of several individual metabolic pathways of yeast, and it is expected that many more will be produced in the future in our laboratory and others. Despite these experimental efforts it is clear that not all enzymes of an organism will be possible to assay *in vitro* (or even *in vivo*). This means that to create a full-genome metabolic kinetic model a different strategy must be applied.

In order to overcome the obstacles described above, kinetic models of large-scale metabolic networks require the use of generic rate laws that can be applied for several different reaction types. These are empirical rate laws that should be able to describe the changes in rates of reaction in terms of the concentrations of its metabolites in an approximate way. Absolute precision is not possible, but the generic behaviour is expected to be captured in these rate laws. We have studied several types of these generic rate laws, such as mass action, lin-log, and convenience kinetics. The lin-log approach is the method that requires the least amount of effort and indeed it is feasible for this approach [4, 5] given a set of measurements of steady state concentrations of metabolites. However this method has poor extrapolation power away from the state used for calibration. Generic rate laws that display saturation are more likely to extrapolate to wider range of conditions and we have been using the convenience kinetics approach.

The convenience kinetics rate law has a number of parameters that need to be estimated for each enzyme. An important class of parameters that need special consideration are the equilibrium constants of each reaction. These are constrained by the structure of the metabolic network: the overall equilibrium constants of two parallel metabolic routes (*i.e.* that start and end in common points, but that use different sets of reactions) must be the same. A special constrained optimisation approach allows us the entire set of equilibrium constants such that they are consisted with each other. By using the values of some equilibrium con-

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stants determined with precision (*i.e.* experimentally) for a few reactions, the other equilibrium constants can also be estimated. This then leaves two other classes of parameters: affinity constants (Michaelis constants, inhibition or activation constants) and limiting rates (V_m) which are estimated by a global fit using known values of fluxes and concentrations of metabolites. This approach produces a large-scale kinetic model of metabolism which should be seen as a hypothesis (or collection of hypotheses) about the dynamic behaviour of the metabolic network. It is important to recognize that such a model is very rough and has low "dynamic resolution" but is nevertheless an important starting point for further improvements. We have started to make our large-scale model of yeast more accurate by substituting the generic rate laws for precise rate laws for those enzymes that we have already studied in detail. Rounds of sensitivity analysis and experimentation are proposed to identify the enzymes that if studied in detail can best improved the accuracy of the model. The approximate low-accuracy model developed is therefore an important piece towards accurate large-scale metabolic models through this strategy proposed here.

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