Perspectives on Genome Scale Modelling of Metabolism

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Genome–Scale Metabolic Models



Genes to Reactions — More Detail

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 Genome-scale metabolic models (GSMs) are arguably the only successful example of predicting a complex (i.e. polygenic) phentoype from a genome sequence.

• Metabolic phenotypes are composed of :

- metabolic capabilities (major nutrients used, products formed);
- nutritional requirements (essential nutrients for growth);
- flux patterns in the metabolic network (pathways used);
- metabolic responses to change in the environment, and
- metabolic effects if any of gene mutations.

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There are two main types of metabolic model:

- Structural need list of reactions with a full stoichiometric
 chemical equation; give existence and number of routes; optimal stoichiometries; network flux values.
- Dynamic or Kinetic need full kinetic description of each enzyme/step; predict time-courses, steady-states, sensitivity analysis or control distribution . . . Can be deterministic or stochastic.

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There is no organism for which we have a complete kinetic description of every metabolic enzyme, hence GSMs are of necessity, structural models.

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There is no organism for which we have a complete kinetic description of every metabolic enzyme, hence GSMs are of necessity, structural models.

The most practical technique to analyse GSMs is **linear programming**, also known as Flux Balance Analysis (FBA) in this context.

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- *Linear Programming (LP)* is a mathematical optimisation method for systems of under–determined linear equations that assigns values to variables, given some known constraints, such that a linear function of some variables is optimised (minimised or maximised).
- *Flux Balance Analysis (FBA)* is the application of LP to structural metabolic models, such that:
 - Reactions (variables) are assigned fluxes (values) consistent with every internal metabolite being at steady state,
- some variables are constrained to match experimental observations and known biochemical limitations,
- and some biologically meaningful objective is optimised.

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Variables: Unknown fluxes.

Constraints: Input and/or output fluxes.

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 $dA/dt = A_t x - R_1$ $dB/dt = R_1 - R_2 - R_4$ $dC/dt = R_2 - R_3$ $dD/dt = R_4 - R_6 - R_5$ $dE/dt = R_3 - R_5 - E_t x$ $dF/dt = R_6$

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 $\mathbf{N}\mathbf{v} = \mathbf{0}$

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Other possible constraints:

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- Other possible constraints:
 - Reaction reversibility, $v_i \geq 0, v_i \leq 0, -\infty \leq v_i \leq \infty$

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- Other possible constraints:
 - Reaction reversibility, $v_i \geq 0, v_i \leq 0, -\infty \leq v_i \leq \infty$
 - \circ Enzyme concentration and catalytic capacity, $|v_i| \leq v_{max}$

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Two ways of representing biomass synthesis. In both cases, major biomass components are assumed to be exported by the metabolic network.

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A common practice is to represent the formation of biomass for growth as a pseudo-reaction of the model with non-integral stoichiometries for each of the biomass components.

• Synthesis is represented as a single reaction where components of biomass are the reactants and their *in vivo* concentrations are represented as stoichiometric coefficients:

(0.000188) 12dgr2_ST + (0.05) 5mthf +
(59.964348) atp ... + (0.284577) val-L -->
x_Bio + (59.81) adp + (58.266936) h + (59.81)
pi + (0.771532) ppi

- It takes what is a variable output of the metabolism and makes it part of the metabolic network structure, so biomass composition cannot be varied as part of the model analysis.
- Biomass composition is known to be variable with, e.g. growth rate of microorganisms.

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- 5mthf_tx: 5mthf --> x_5mthf, # v_{5mthf_tx} = $0.05 \cdot \mu$

- Biomass composition can be changed without changing the model structure.
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Applications of FBA are often said to involve maximisation of growth rate.

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Max.

subject to $\begin{cases} \mathbf{Nv} = \mathbf{0} & \leftarrow \text{steady state constraint} \\ v_{qlucose} \leq v_{obs} & \leftarrow \text{Glucose uptake limited} \end{cases}$

where:

biomass: (0.000188) 12dgr2_ST + ... + (0.284577) val-L --> x_Bio + (59.81) adp + (58.266936) h + (59.81) pi + (0.771532) ppi glucose: x_glucose --> glucose

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where:

- biomass: (0.000188) 12dgr2_ST + ... + (0.284577) val-L --> x_Bio + (59.81) adp + (58.266936) h + (59.81) pi + (0.771532) ppi
- glucose: x_glucose --> glucose

In fact, this is maximum biomass yield for a fixed amount of substrate. (Schuster et al, J Theor Biol, 252, 497–504 2008)









The predicted growth rate is the yield times the substrate uptake rate.



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- Minimisation of enzyme investment, modelled as minimisation of flux.
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Examples of objectives used in FBA:

- Minimisation of enzyme investment, modelled as minimisation of flux.
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- Maximisation of ATP yield.

Note that, without including any mechanisms of regulation in an FBA model, the objective function introduces implicit metabolic regulation.

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Note that, without including any mechanisms of regulation in an FBA model, the objective function introduces implicit metabolic regulation. But what objective is appropriate or a non–growing cell of a tissue in a multi–cellular organism?

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Does it matter? Surely any maximisation can be expressed as a minimisation and vice versa?

On maximisation, LP algorithms drive all variables as far as possible to their upper limits (constraints), which requires that upper bounds are placed on fluxes. As a result, solutions contain maximal fluxes for any non–driven cycles, requiring complicated post–processing to eliminate this artefact.

Minimisation of substrate input for a given amount of biomass would give essentially the same solution, but the algorithm would drive variable values as low as possible, so non-driven cycles are assigned zero net flux.

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- The objective (originally proposed by Holzhütter) is to mimimise the sum of all fluxes in the system for consumption of a given amount of nutrient, implying minimum investment in enzymic machinery.
- This allows assignment of different costs to different reactions to represent other constraints, such as protein synthesis cost of different enzymes, or investment of scarce resources such as iron.
 - In a comparison of FBA solutions of our Arabidopsis model with flux estimates by 13 C Metabolic Flux Analysis, minimisation of total flux gave the best match. (*Cheung et al, Plant Journal, 75* 1050–61, 2013)

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- All cells consume substrates for maintenance even when they are not growing.
 - For microbes, chemostat experiments can determine the non-growth associated substrate requirement. This is usually converted to an ATP requirement, according to the ATP yield of complete catabolism of the substrate.
 - In FBA models, this maintenance is represented by a constraint on a generic ATPase reaction, which is needed anyway because creating biomass generates excess ATP. But is the same constraint valid in all circumstances, and is the substrate requirement solely about ATP generation?

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• No, maintenance is not constant, and it's not just ATP generation.

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• No, maintenance is not constant, and it's not just ATP generation.

• Even for *E. coli*, maintenance varies several fold depending on the limiting substrate.

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• No, maintenance is not constant, and it's not just ATP generation.

• Even for *E. coli*, maintenance varies several fold depending on the limiting substrate.

For aerobic organisms, some maintenance is combating oxidative stress by making NADPH. In the comparison of FBA solutions of our Arabidopsis model with flux estimates by ¹³C Metabolic Flux Analysis, the pentose phosphate pathway fluxes could only be matched by assuming a significant production of NADPH. (*Cheung et al, Plant Journal, 75 1050–61, 2013*)

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The potential causes could have been:

- Transhydrogenase cycles in the model solutions causing generation of NADPH from NADH via dehydrogenases with dual specificity for NAD and NADP or pairs of NAD– and NADP– specific enzymes for the same metabolic conversion.
- Lack of sufficient representation of compartmentation in the original model allowing transhydrogenase cycles that would be prevented by physical compartmentation in the cell, or NADPH generated in one compartment being utilised in another.
- Under-estimation of the requirement for NADPH generation, e.g. for cell maintenance in the face of oxidative stress.

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We investigated the effect of a requirement for additional NADPH in two ways:

- We did a 2–D scan of two constraints: ATP demand and NADPH demand. We found the locus of glucose uptake equal to the experimental uptake; on that line we found a point where the ratio of glycolysis:OPPP fluxes was the same as in the MFA model.
 - We constrained the glucose uptake to the experimentally observed value, then maximised the ATP and NADPH generation using a range of relative weighting factors to obtain the Pareto optimal front for dual optimisation of ATP and NADPH. Again, on this front, we located the point matching the MFA result.

The outcome is similar in both cases.
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Point 1 coincides with the flux distribution in the MFA solutions.

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Distances between the model flux solutions and the MFA result for different optimisation functions.

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Many metabolic models (including Human Recon2) contain the reaction:

succinate + FAD \leftrightarrow fumarate + FADH₂

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This is WRONG! It's an old textbook error.

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- This is **WRONG!** It's an old textbook error.
- There is no pool of circulating FAD, nor is FADH₂ the product of the succinate dehydrogenase reaction, nor of any of the other flavin–containing dehydrogenases. It remains bound to its enzyme and participates in the catalytic cycle.

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- There is no pool of circulating FAD, nor is FADH₂ the product of the succinate dehydrogenase reaction, nor of any of the other flavin–containing dehydrogenases. It remains bound to its enzyme and participates in the catalytic cycle.
- The correct form of the reaction is:

succinate + CoQ \leftrightarrow fumarate + CoQH₂

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Take a model and try the following test:

• Set the transport rate of all carbon–containing substrates to zero, and the rate of hydrolysis of ATP to a positive value.

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There are published models that fail this fundamental test.

There should also be no solutions for net oxidation or reduction of NAD(P)H.

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- Is bigger always better?
- Thermodynamics avoiding futile cycles
- Database errors
- Stoichiometric testing
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- Incorporating experimental data
- Situations where growth is not an appropriate objective
 - multicellular organisms, microbial communities.

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- FBA is a powerful tool for modelling metabolism and exploiting genome sequences ...
- but some of the current methodologies and assumptions should be critically assessed for appropriateness in different situations.
- Often it is best regarded as a means of generating hypotheses about the functioning of metabolic networks.

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