Integrated Flux Balance Analysis (iFBA)

Metabolische Netzwerke SS 15

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Integrated Flux Balance Analysis (iFBA)

Covert/Xiao/Chen/Karr'08

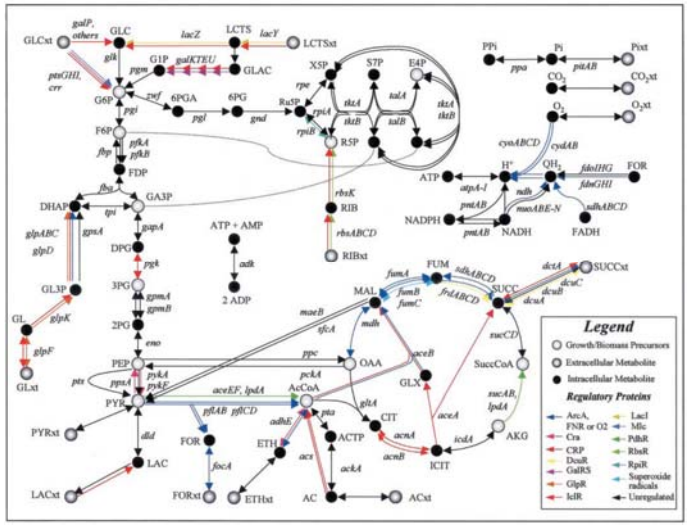
"A major advantage of rFBA - requiring few kinetic parameters - could be a weakness in situations where the kinetic parameters have been determined and capture information not contained in rFBA."

- Integrate available kinetic information into rFBA models (iFBA).
- More precisely: combine rFBA and ODE model by identifying values to pass from either model to the other.

Software available:
http://covertlab.stanford.edu/projects/iFBA/

rFBA model of central metabolism in E.coli

Covert/ Palsson'02



ODE model of *E.Coli* carbohydrate uptake

Kremling et al.07

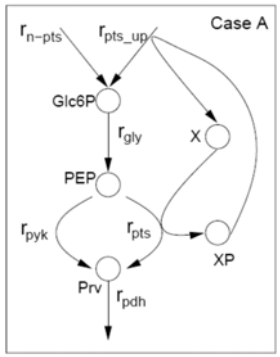
Differential equations

$$\dot{G6P} = r_{n-pts} + r_{pts\_up} - r_{gly}$$

$$\dot{PEP} = 2r_{gly} - r_{pyk} - r_{pts}$$

$$\dot{Prv} = r_{pts} + r_{pyk} - r_{pdh}$$

$$\dot{XP} = r_{pts} - r_{pts\_up}$$



Rate laws

$$r_{gly} = k_{gly}\, G6P$$

$$r_{pdh} = k_{pdh}\, Prv$$

$$r_{pts} = k_{pts}(PEP(X_0 - XP) - K_{pts}\, Prv\, XP)$$

$$r_{pyk} = k_{pyk}\, PEP\, f\,(PEP, \ldots),$$

Steady-state conditions

$$r_{pts} = r_{pts\_up}$$

$$r_{gly} = r_{n\text{-}pts} + r_{pts\_up}$$

$$r_{pdh} = 2\,(r_{n\text{-}pts} + r_{pts\_up})$$

$$r_{pyk} = 2\,r_{n\text{-}pts} + r_{pts\_up}$$

Steady-state concentrations

Kremling et al.07

$$G6P = \frac{r_{n-pts} + r_{pts\_up}}{k_{gly}}$$

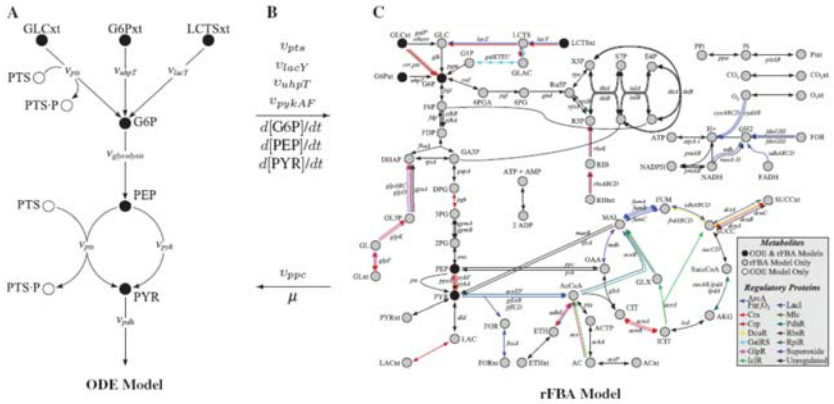
$$Prv = 2\frac{r_{n-pts} + r_{pts\_up}}{k_{pdh}}$$

$$PEP = \frac{2r_{n-pts} + r_{pts\_up}}{k_{pyk}f}$$

$$XP = k_{pdh}\frac{X_0 - \frac{r_{pts\_up}}{k_{pts}PEP}}{k_{pdh} + 2K_{pts}k_{pyk}f\frac{r_{n-pts} + r_{pts\_up}}{2r_{n-pts} + r_{pts\_up}}}$$

Integration of ODE and rFBA model

Covert/Xiao/Chen/Karr'08

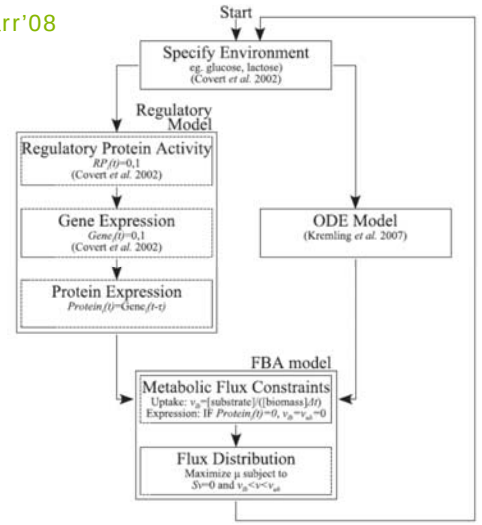


Pass values from one model to the other

- Identify metabolites and fluxes common to both models (black circles in Fig.)
- Variables passed from the ODE model:
  - enzyme fluxes v_{pts}, v_{lacY}, v_{uhpT}, v_{pykAF}
  - changes in metabolite concentrations d[G6P]/dt, d[PEP]/dt, d[PYR]/dt
- Variables passed from the rFBA model:
  - growth flux μ
  - flux through phosphoenolpyruvate carboxylase v_{ppc}

iFBA algorithm

Covert/Xiao/Chen/Karr'08



iFBA algorithm

1. Specify initial environment
2. Calculate regulatory protein activity, gene and protein expression
   - Boolean regulatory model with time delays (Covert et al. 2001)
   - some Boolean regulatory values superseded by ODE model: Crp, *galEKMPT*, *lacYZ*, *pgk*, *ptsG*, and *pykF*
3. Solve ODEs
   - At each time step, numerically integrate the ODE model using the growth rate and *ppc* flux computed by the FBA model at the previous time step.
   - Calculate ODE rates at the end of the time step to later constrain the FBA linear program.

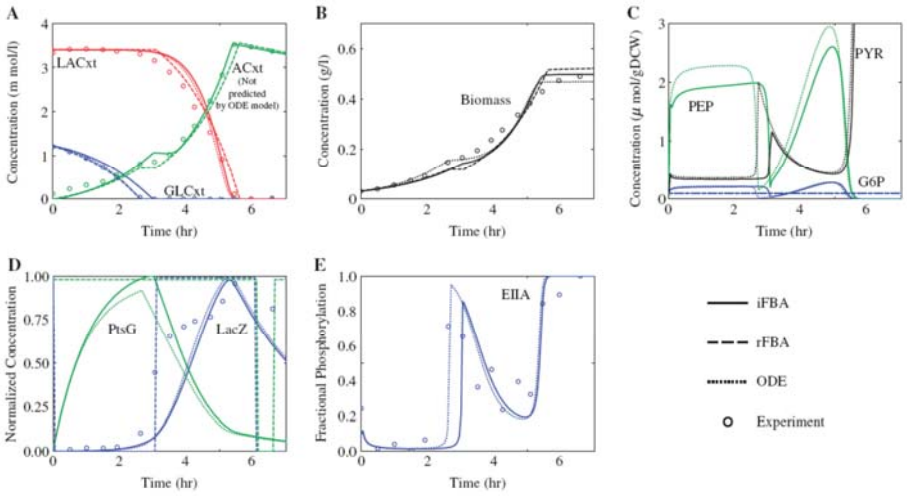
iFBA algorithm

4. Determine metabolic flux constraints and metabolite pooling fluxes
   - Flux constraints:
     - thermodynamic irreversibility
     - environment: available amount of substrate in the culture medium
     - transport: maximum substrate uptake or by-product secretion rate
     - regulation: restrict flux through an enzyme according to the expression of the corresponding protein(s)
     - ODE matching: specification of fluxes by the ODE model
   - Set rhs of FBA linear program to concentrations calculated by ODE model: d[G6P]/dt, d[PEP]/dt, d[PYR]/dt
5. Calculate flux distribution: Maximize biomass production
6. Calculate new environment: Use growth rate and fluxes computed by the FBA model to update biomass and metabolite concentrations

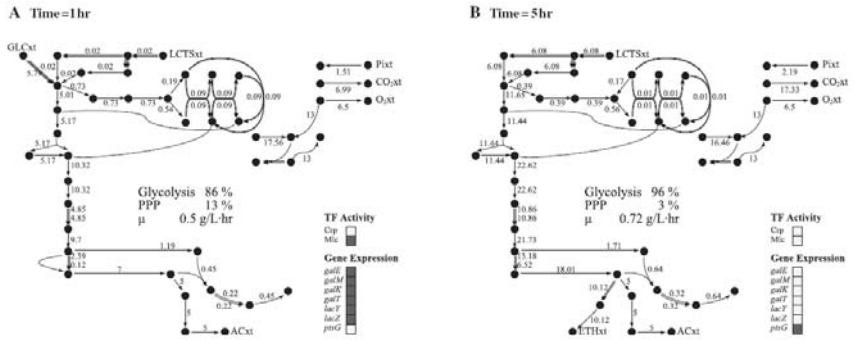
$$[\text{biomass}](t+\Delta t) = \beta[\text{biomass}](t)e^{\mu\Delta t}$$

$$[\text{met}_i](t+\Delta t) = [\text{met}_i] + \frac{v_{ex}}{\mu}[\text{biomass}](t)\left(1-e^{\mu\Delta t}\right)$$

Comparing the three approaches



Changes in gene expression and flux distribution

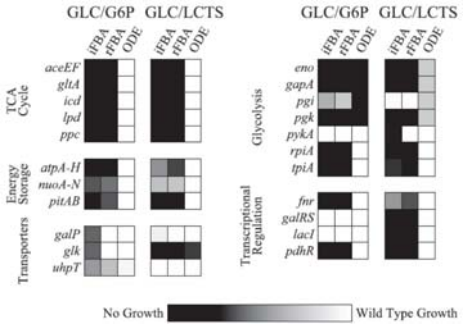


- bacteria are consuming glucose
- significant flux from internal glucose- 6-phosphate through the pentose phosphate pathway

- bacteria are consuming lactose
- flux from glucose-6-phosphate has shifted towards glycolysis
- the lactose-related TFs GalE, GalM, GalK, and GalT are now expressed, while PtsG is suppressed and bacteria secrete ethanol in addition to acetate

Single gene perturbation

- 165 single gene perturbations
- iFBA predicts different phenotypes than ODE for 41 resp. 45 of the mutants on glucose/glucose-6-phosphate resp. glucose/ lactose.
- Corresponding genes can be grouped into 5 classes (see Fig.)
- In most of the 85 cases, iFBA and rFBA predicted the correct phenotype, while ODE failed.
- In 2 cases, IFBA predicted different phenotypes than rFBA (better account of the dynamics of internal metabolites).



Discussion

- Kinetic description in iFBA much more detailed than in rFBA.
- Concentration of internal metabolites not calculable without kinetic model (rFBA uses approximation from external metabolite concentrations or combinations of fluxes).
- iFBA can model enzymes that do not contribute to metabolic growth, but have another important function such as signal transduction ("dead ends" in standard FBA).
- Compared with ODE, iFBA may compute flux distributions for an entire network with only a few parameters -> more predictive power than ODE, both in terms of scope and accuracy.
- ODE models can capture intracellular concentration and short-time dynamics (critical in signal transduction) -> iFBA as an integrating framework.

Possible improvements:

- More accurate objective functions
- Flux variability analysis

References

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