Using Pathway Tools & Matlab for Flux Balance Analysis

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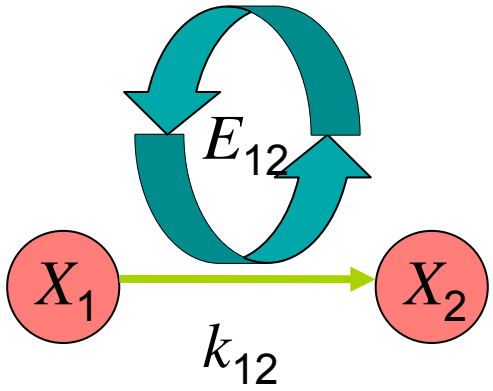
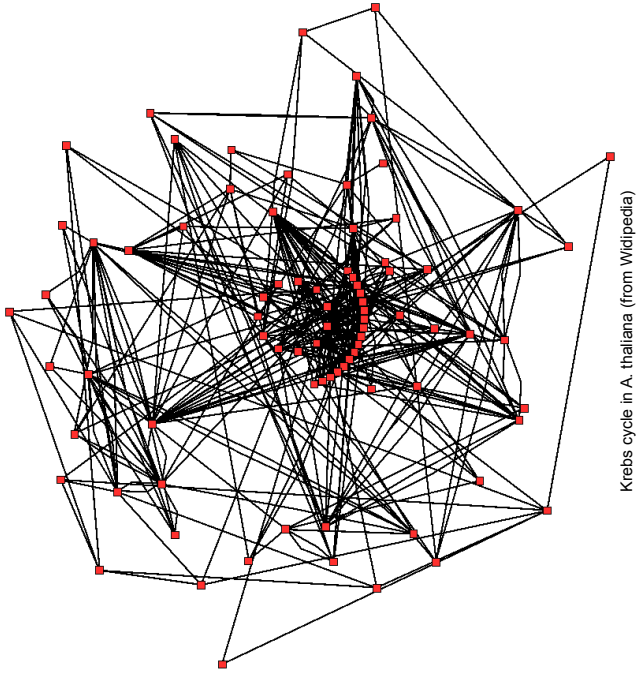
25 Aug. 2009

Summary

- Flux Balance Analysis (FBA)
- Workflow: from Ptools to Matlab, and back again
- A simple example
- What next?

Let's start at the very beginning ...





Bio Chemical Reaction Network

Systems biology:

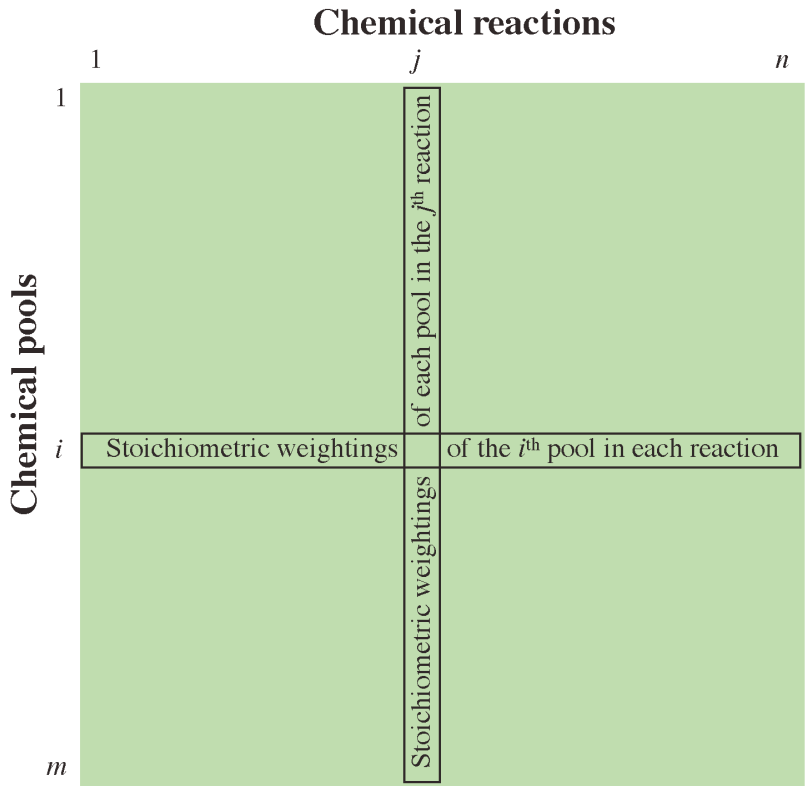
Stoichiometry and kinetics

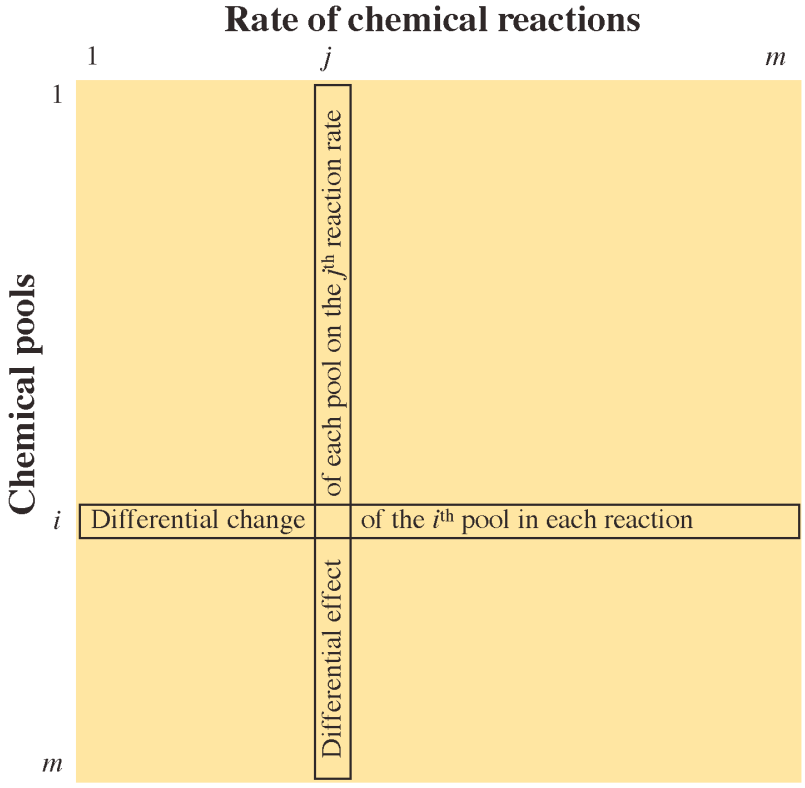
Matrix of stoichiometric weights (S)

$$S_{ij} = \left.\frac{\dot{x}_i}{v_j}\right|_{v_{k\neq j}=0}$$

Matrix of linearized kinetics ($D_x r$)

$$D_x r_{ij} = \left.\frac{r_i}{x_j}\right|_{x_{k\neq j}=0}$$





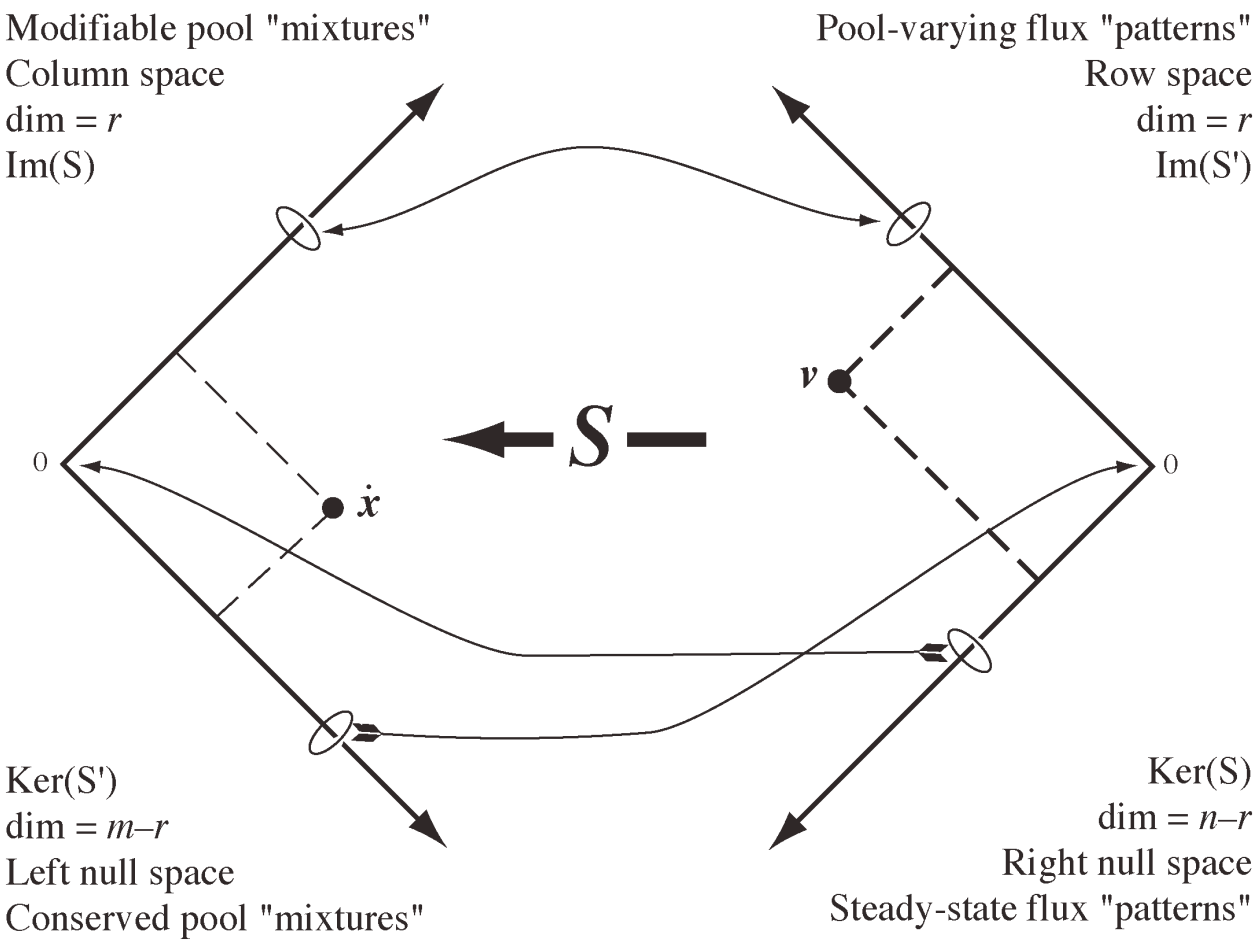
Systems biology:

Stoichiometric (a.k.a. kinematic, structural) constraints

Chemical pool growth rates, $\dot{x}_i$

$$[\dot{x}_i] = [S_{ij}]\,[v_j]$$

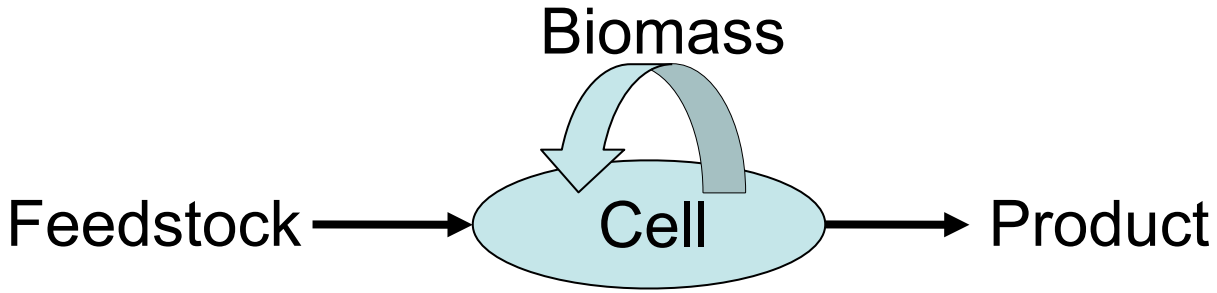
Chemical reaction velocities, v_j



Flux Balance Analysis (FBA)

- Flux = “rate at which chemicals are transformed or transported”
- Balanced = “input & output rates are equal” (a.k.a. steady-state)
- Analysis = “optimize (balanced) flux with constraints”

- Example: maximize product synthesis per unit feedstock,
  while satisfying maintenance (biomass) requirements



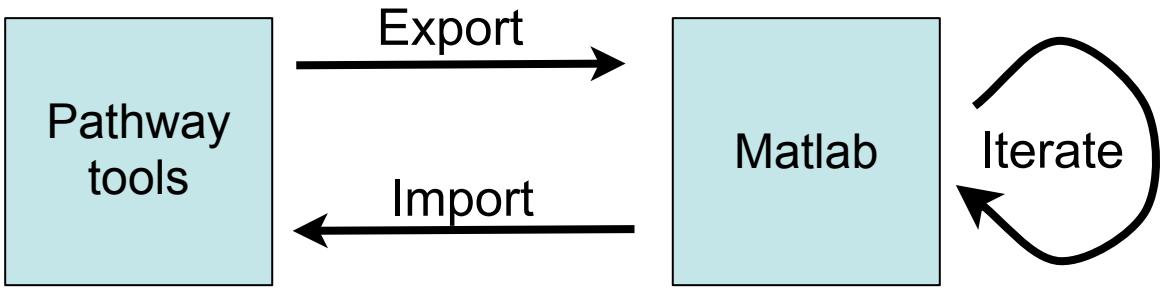
Software components

- Pathway Tools[1] (PGDB for organism)
- SBML[2] Toolbox (export metabolic description files)
- COBRA[3] (setup the LP)
- GLPK[4] (solve the LP)
- Matlab[5] (math's programming & graphics)

1. http://bioinformatics.ai.sri.com/ptools/
2. http://sbml.org/Software/SBMLToolbox
3. Palsson lab @ UCSD, http://gcrg.ucsd.edu/Downloads/Cobra_Toolbox
4. GNU Linear Programming Kit, http://glpkmex.sourceforge.net/
5. http://www.mathworks.com/

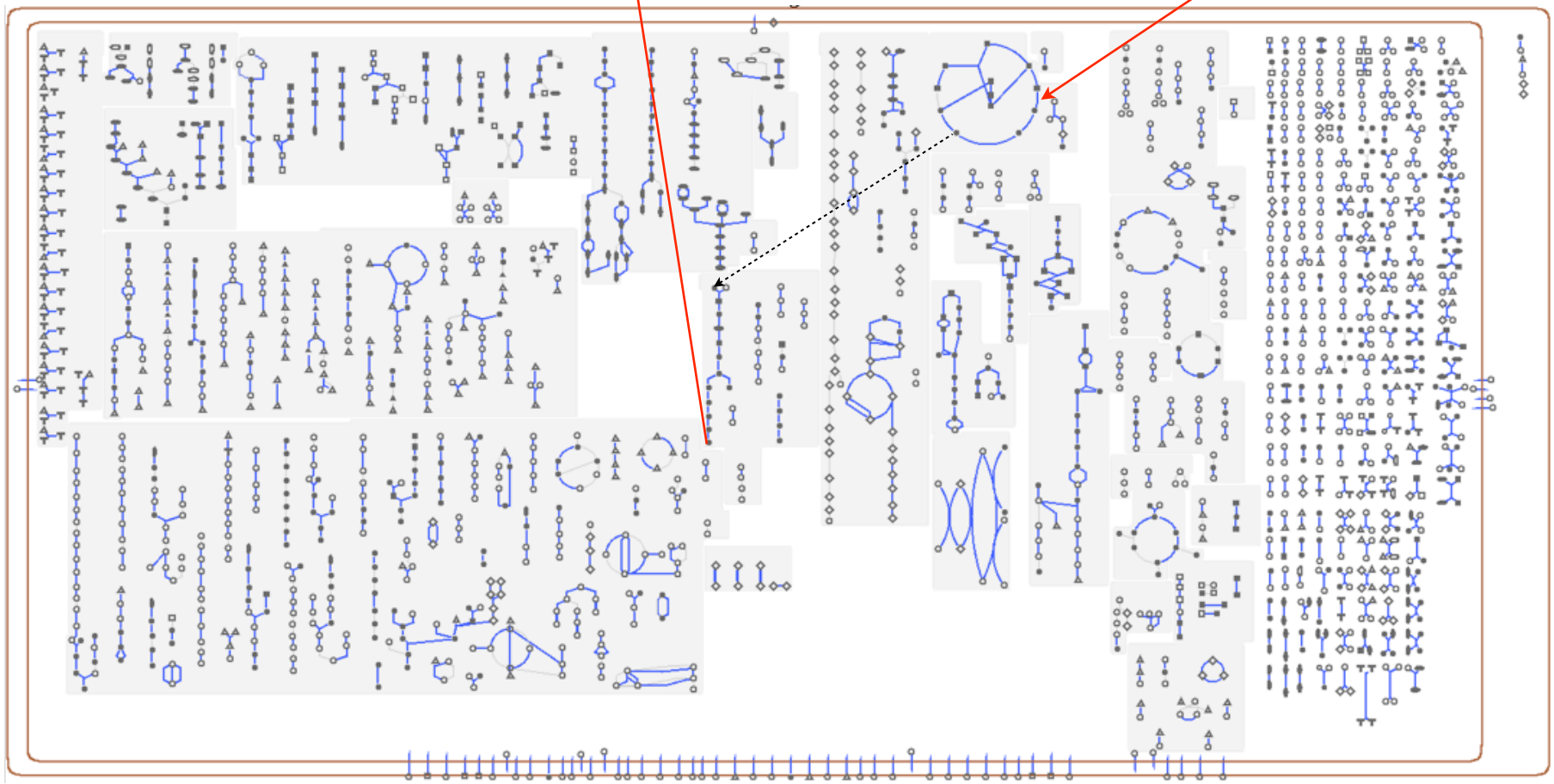
Workflow

- Load PGDB into PathwayTools
- Export metabolic network into SBML file
- Import SBML file into Matlab
- Compute FBA solution in Matlab
- Examine network & flux sol'n, using Matlab GUIs
- Iterate
- Write FBA sol'n to text file
- Import sol'n to Pathway Tools Omics Viewer



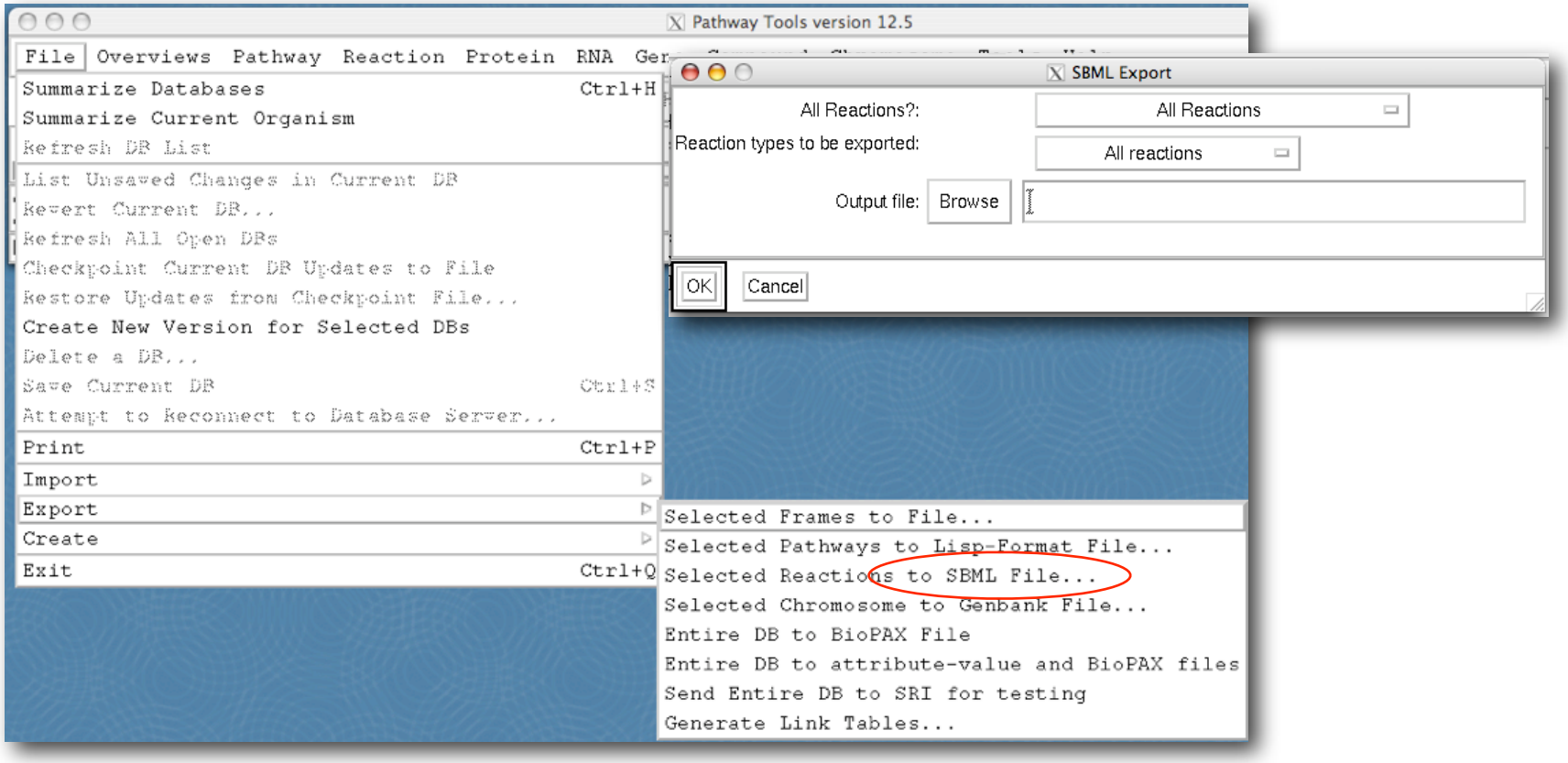
Example

Maximize anabolic “biomass” produced from a “feedstock”

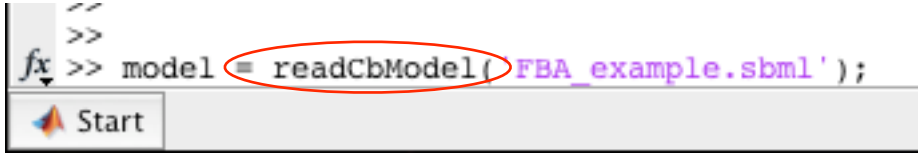


Getting the data from Ptools to Matlab

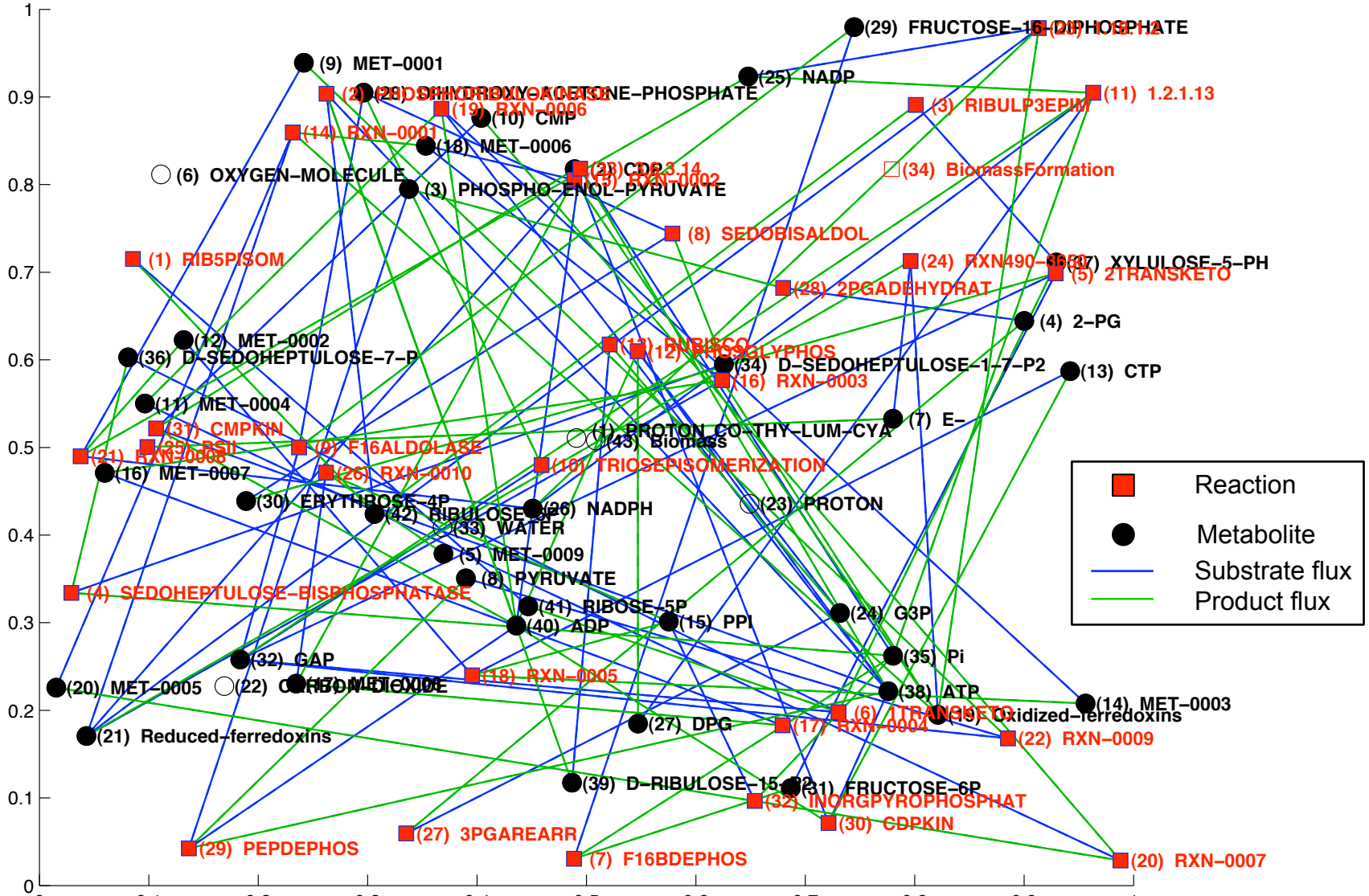
- SBML export from Pathway Tools



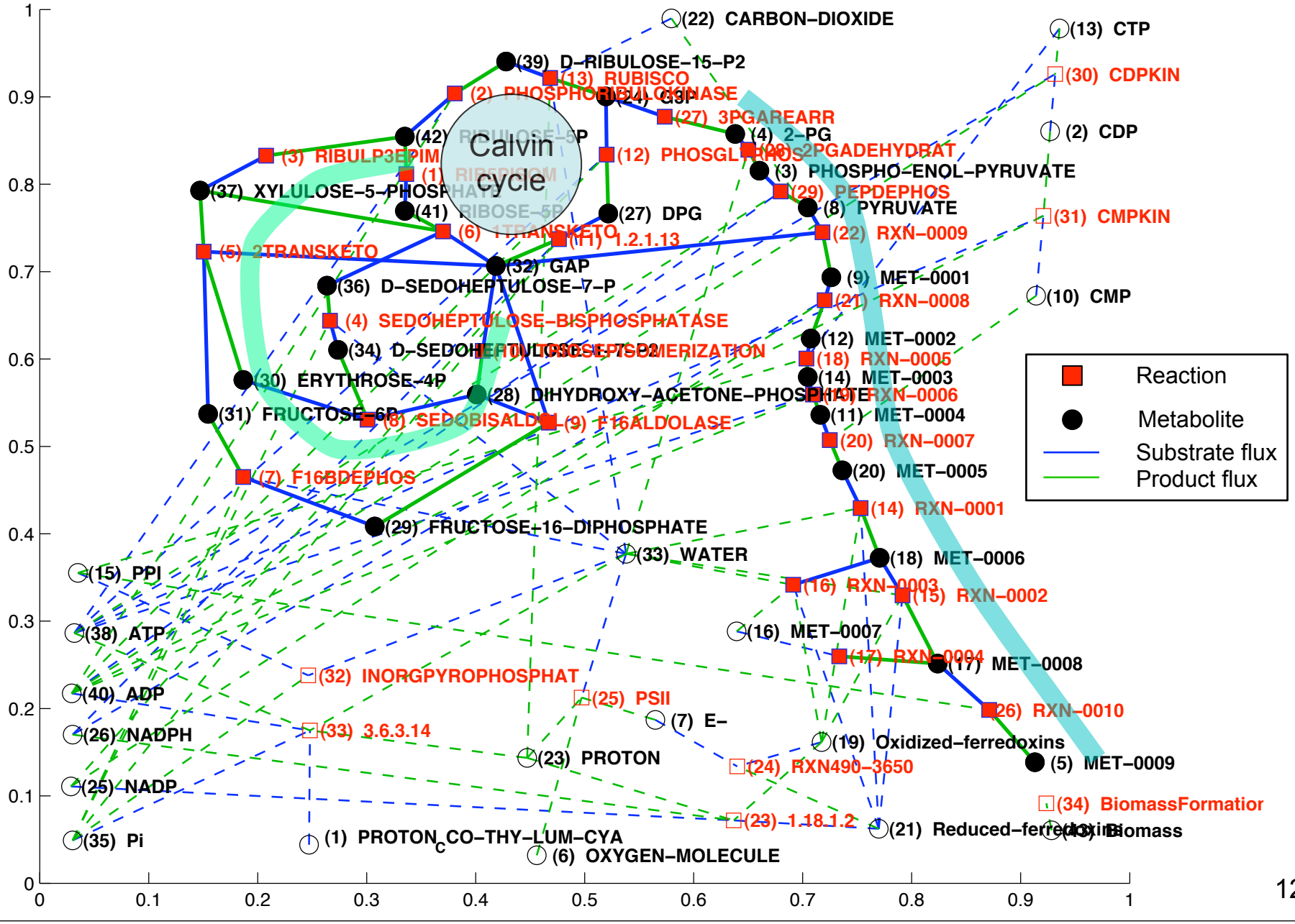
- SBML import into Matlab



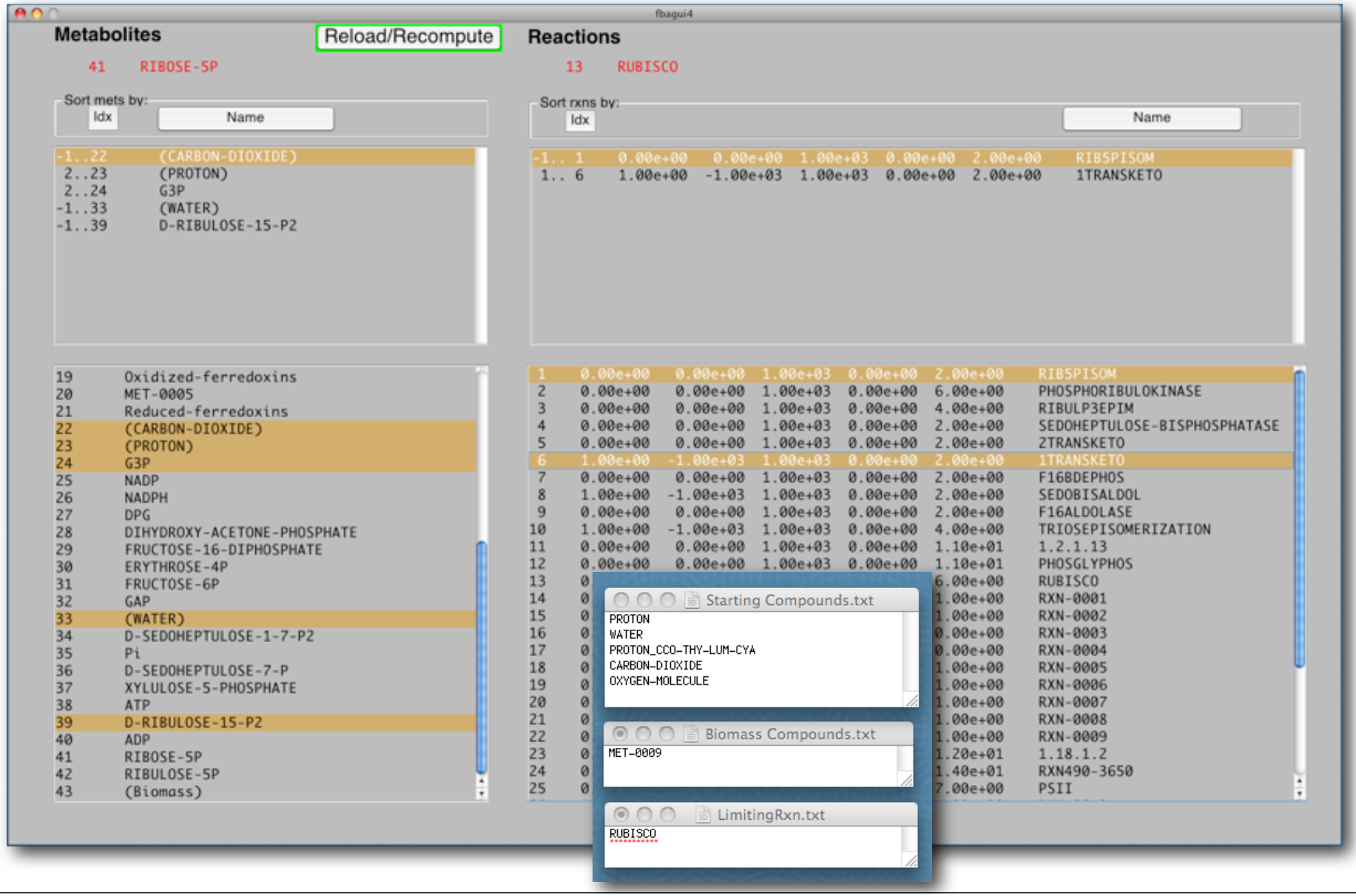
What a mess!


(29) FRUCTOSE-16-DIPHOSPHATE
(9) MET-0001
(25) NADP
(11) 1.2.1.13
(3) RIBULP3EPIM
(19) RXN-0006
(10) CMP
(14) RXN-0001
(18) MET-0006
(6) OXYGEN-MOLECULE
(3) PHOSPHO-ENOL-PYRUVATE
(34) BiomassFormation
(8) SEDOBISALDOL
(1) RIB5PISOM
(24) RXN490-6558
(37) XYLULOSE-5-PH
(5) 2TRANSKETO
(28) 2PGADEHYDRAT
(4) 2-PG
(12) MET-0002
(36) D-SEDOHEPTULOSE-7-P
(34) D-SEDOHEPTULOSE-1-7-P2
(13) CTP
(16) RXN-0003
(11) MET-0004
(31) CMPKIN
(7) E-
(1) PROTON_CO-THY-LUM-CYA
(43) Biomass
(21) RXN-0008
(9) F16ALDOLASE
(16) MET-0007
(26) RXN-0010
(101) TRIOSEPISOMERIZATION
(30) ERYTHROSE-4P
(26) NADPH
(23) PROTON
(42) RIBULOSE-5P
(33) WATER
(5) MET-0009
(8) PYRUVATE
(4) SEDOHEPTULOSE-BISPHOSPHNATASE
(41) RIBOSE-5P
(15) PPI
(24) G3P
(40) ADP
(32) GAP
(35) Pi
(20) MET-0005
(22) CARBON-DIOXIDE
(18) RXN-0005
(38) ATP
(14) MET-0003
(6) TRANSKETO
(19) Oxidized-ferredoxins
(27) DPG
(17) RXN-0004
(22) RXN-0009
(21) Reduced-ferredoxins
(39) D-RIBULOSE-15-P2
(31) FRUCTOSE-6P
(32) INORGPYROPHOSPHAT
(30) CDPKIN
(27) 3PGAREARR
(29) PEPDEPHOS
(7) F16BDEPHOS
(20) RXN-0007
Reaction
Metabolite
Substrate flux
Product flux
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1

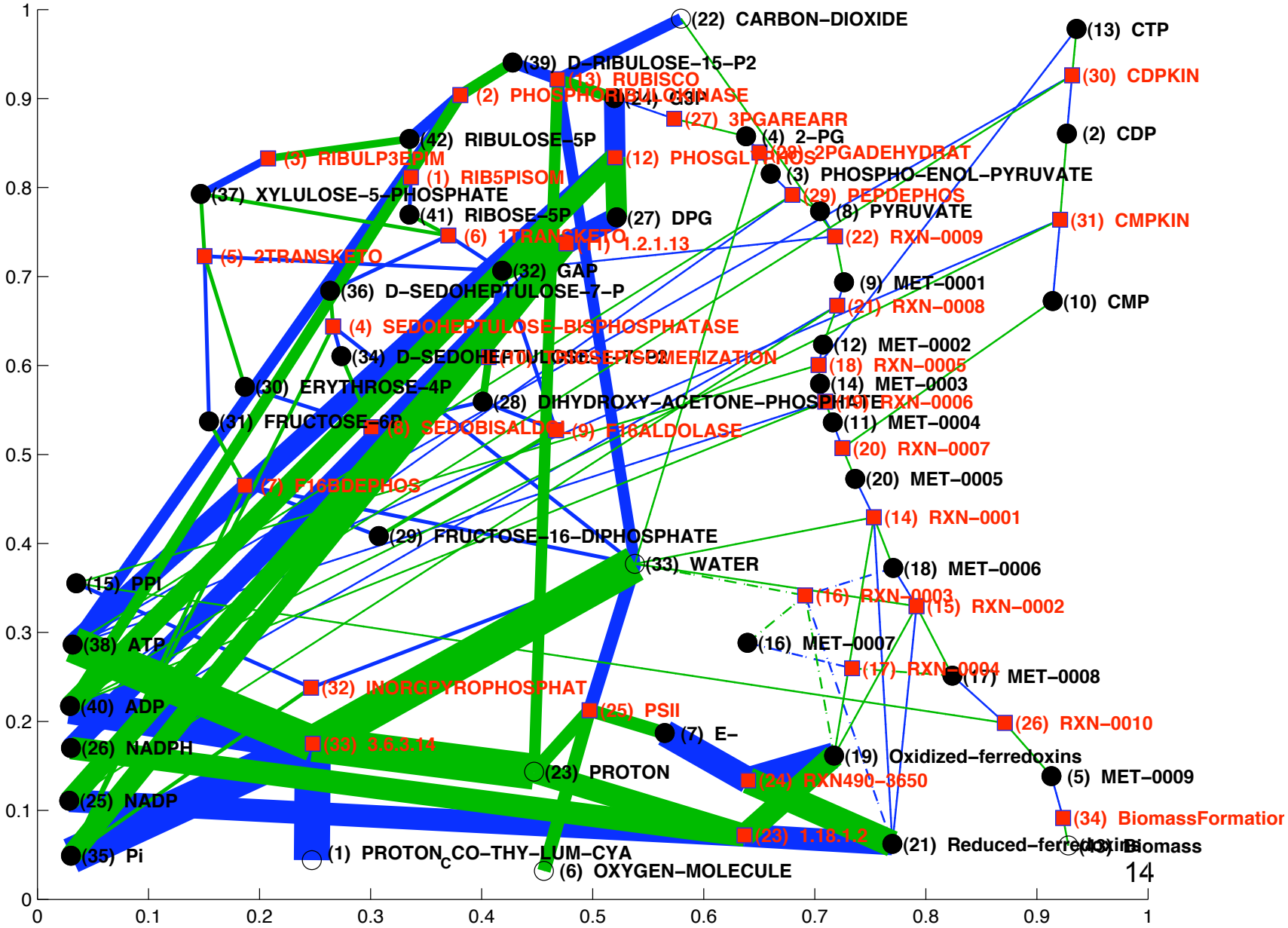
Matlab tools (1): Met/Rxn graph



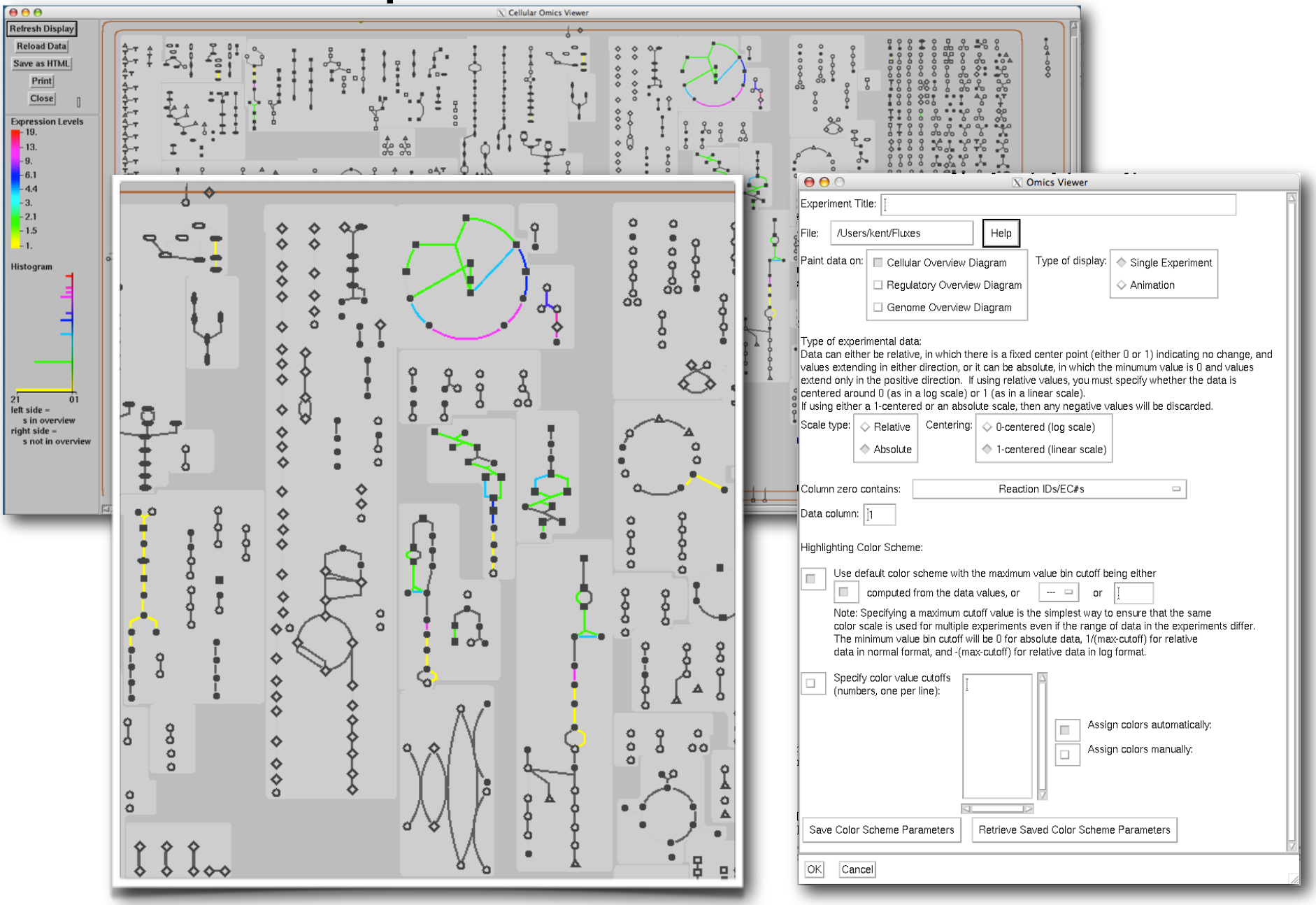
Matlab tools (2): Met/Rxn browser



Final product: an FBA solution



Import to Omics Viewer



Recap

- What have we accomplished?
  - From stoichiometry (structure) of reaction network ...
    - Steady-state flux "subspace", $v \in \mathcal{N}(S)$
    - Conserved "motifs", $\dot{x} \in \mathcal{N}(S^T)$
  - From flux constraints ...
    - Unique steady-state operating point (solution of LP)
    - We used "biomass" optimization, but there are many variations on the theme (e.g., MOMA, ROOM)
- What is not represented?
  - Quantity (Concentrations)
  - Thermodynamics (chemical potentials)
  - Dynamics (approach to steady-state, stability,...)
  - Spatial inhomogeneities
  - Regulation (neither metabolic nor transcriptional)

Systems biology:

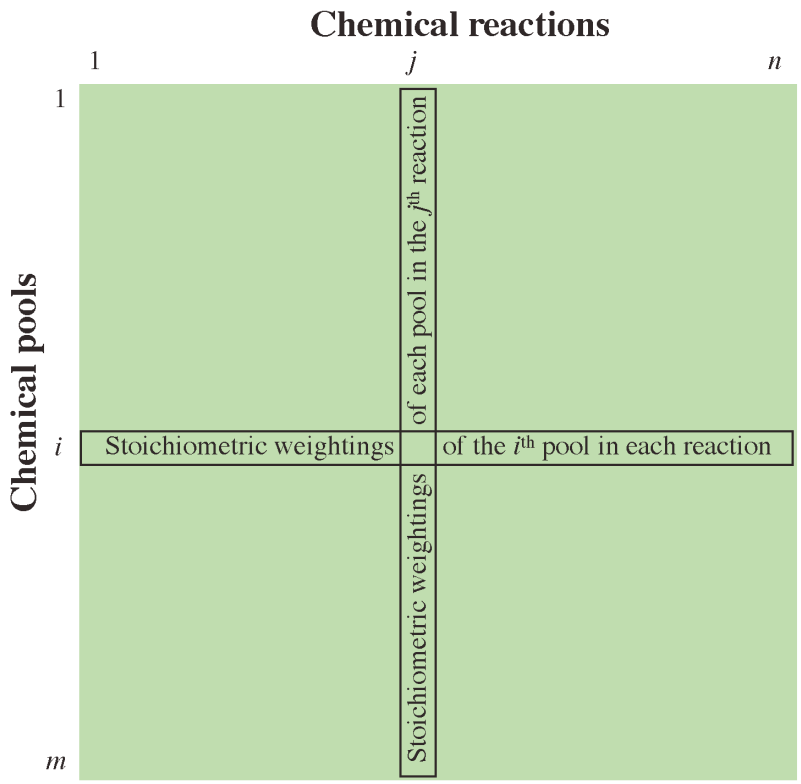
Stoichiometry and kinetics

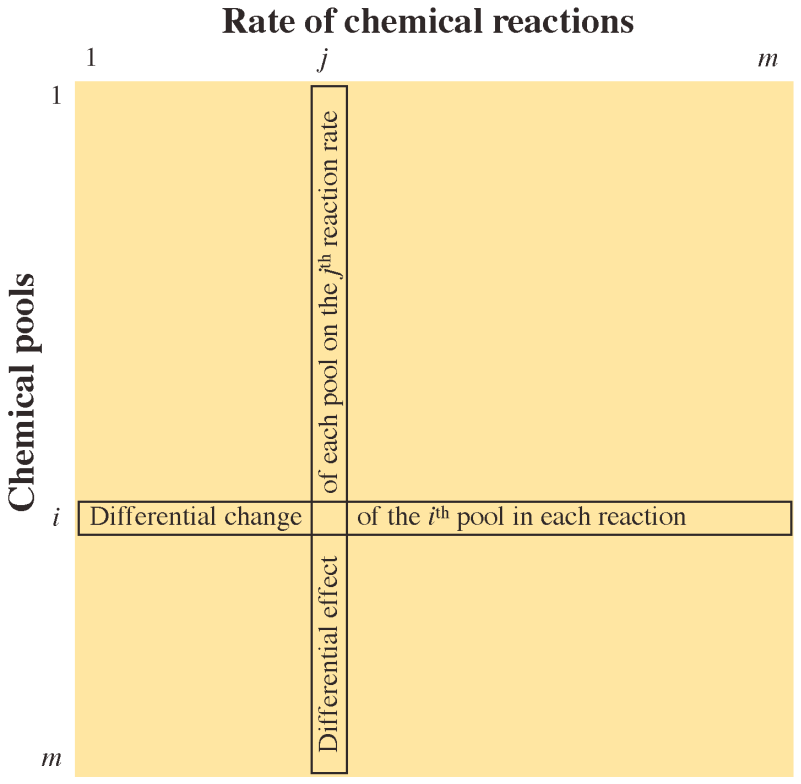
Matrix of stoichiometric weights (S)

$$S_{ij} = \left. \frac{\dot{x}_i}{v_j} \right|_{v_{k \neq j} = 0}$$

Matrix of linearized kinetics ($D_x r$)

$$D_x r_{ij} = \left. \frac{r_i}{x_j} \right|_{x_{k \neq j} = 0}$$

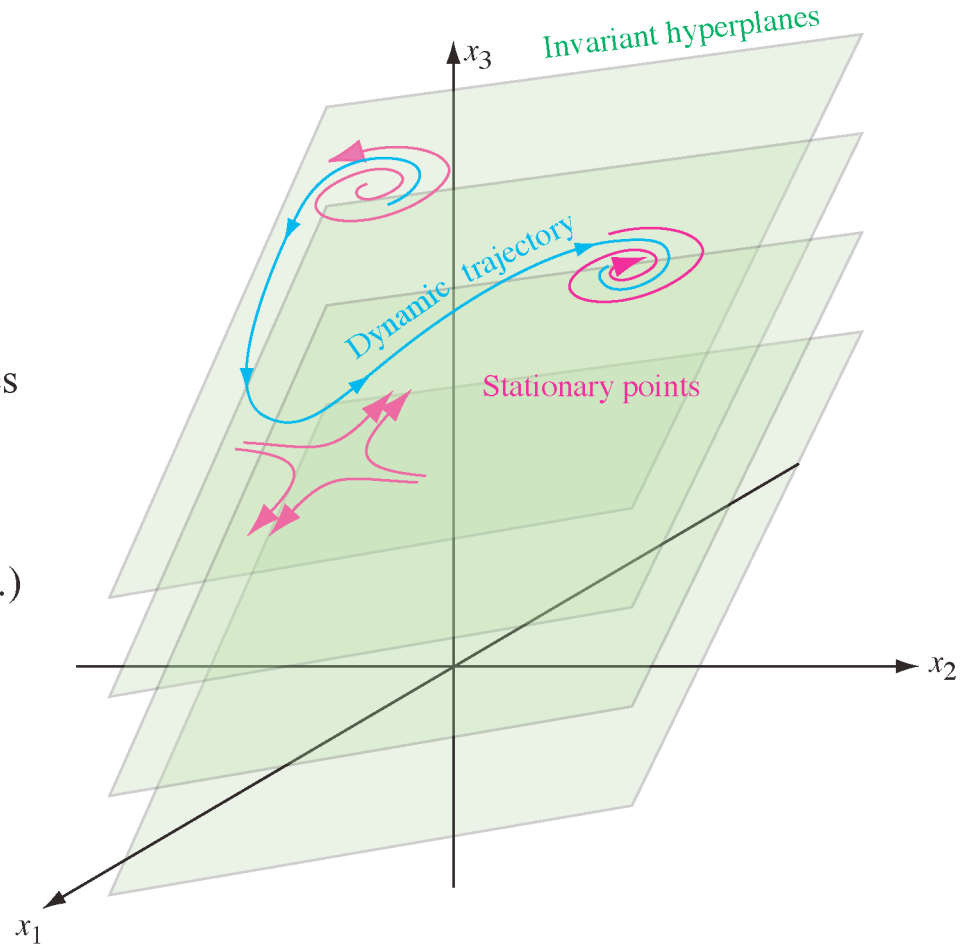




Systems biology:

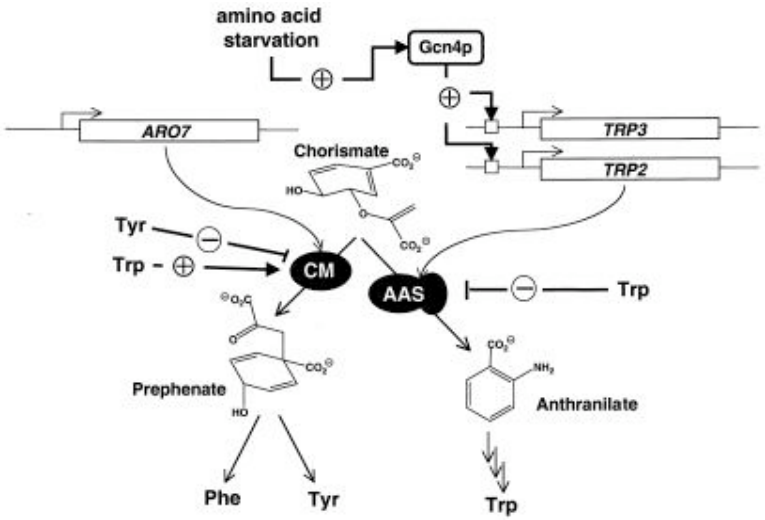
Dynamical behavior of chemical & electrical networks

	Chemical	Electrical
Network:		
State	Chem.pools	Electrical charge
Flow	Accum.rates	Current
Force	Chem.potential	Voltage
Connections	Stoichiometry	Wiring
Init.cond.	Chem.pools	Capacitor charges
Dynamics determined by:		
Constitutive relations	React.kinetics	Device physics (transistors, etc.)
Ext.environ.	Exchange pools	I/O signals/loads
Params.	Regulatory modes	Supervisory modes



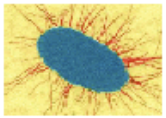
A network model can (should?) include:

- Structure (connections, stoichiometric weights)
- Flux (chemical flow)
- Concentration (chemical potential)
- Kinetics (flux/potential constitutive model)
- Regulatory "feedback"



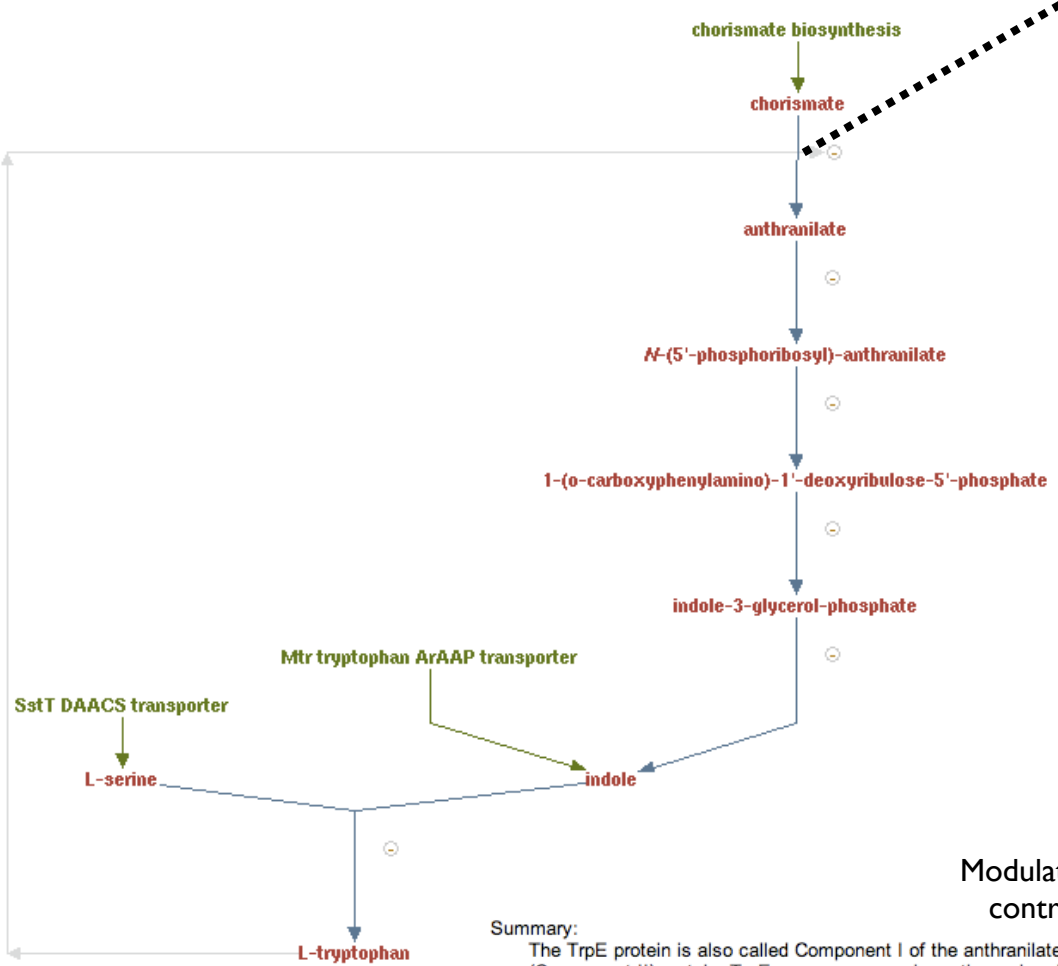
Ref: Krappmann, Lipscomb, Braus (2000) PNAS 97(25): 13585.

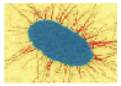
Example of "Metabolic Control" from EcoCyc



E. coli K12 Pathway: tryptophan biosynthesis

More Detail | Less Detail | Cross-Species Comparison | Downloa





E. coli K12 Enzyme: anthranilate synthase

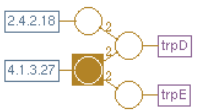
Summary:
The native anthranilate synthase enzyme exists as a tetrameric complex of two subunits each of th
The TrpD protein is bifunctional; it also catalyzes the second reaction in the biosynthesis of tryptop

Citations: [Li74 , Nichols81 , Yanofsky81a]

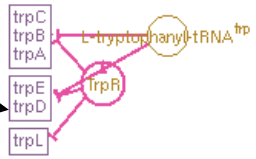
Subunit composition of anthranilate synthase = $[TrpE]_2[TrpD]_2$
anthranilate synthase component I = TrpE
anthranilate synthase component II = TrpD

Genetic Regulation Schematic:

Gene-Reaction Schematic:



Transcriptional control



MultiFun Terms: location of gene products -> cytoplasm
metabolism -> biosynthesis of building blocks -> amino acids -> tryptophan

Enzymatic reaction of: anthranilate synthase

Synonyms: ASase

chorismate + L-glutamine <=> anthranilate + pyruvate + L-glutamate

The reaction direction shown, that is, A + B <==> C + D versus C + D <==> A + B, is in accordance wi

This reaction is reversible.

Alternative Substrates for L-glutamine: ammonia

In Pathways: tryptophan biosynthesis

Summary:
The complex of the TrpE (Component I) and TrpD (Component II) proteins uses either glutamine or contains the binding site for chorismate. In the absence of TrpD it cannot catalyze the formation of can form anthranilate using ammonia instead of glutamine as the amino donor [Ito69]. Anthranilate responsive to changes in the free tryptophan concentration [Nichols81].

Citations: [Nichols81 , Crawford89 , Ito69a]

Cofactors: Mg^{2+} [Ito69b]

Alternative Cofactors for Mg^{2+}: Co^{2+}, Fe^{2+}

Inhibitors (Competitive): L-tryptophan [Comment 1]

Inhibitors (Noncompetitive): L-tryptophan [Comment 2]

Primary Physiological Regulators of Enzyme Activity: L-tryptophan

Modulatory control

Summary:
The TrpE protein is also called Component I of the anthranilate synthase enzyme complex. *In vitro* in the absence of the TrpD (Component II) protein, TrpE can use ammonia as the amino donor for the synthesis of anthranilate from chorismate at approximately 20% efficiency [Ito69]. As a component of the anthranilate synthase complex, TrpD (Component II) provides the glutamine amidotransferase function that allows glutamine to serve as the amino donor in anthranilate formation.

The TrpE subunit contains the tryptophan binding site for feedback inhibition [Crawford89]. Both the TrpE and TrpA polypeptides in the trp operon lack tryptophan residues [Nichols81 , Nichols81].