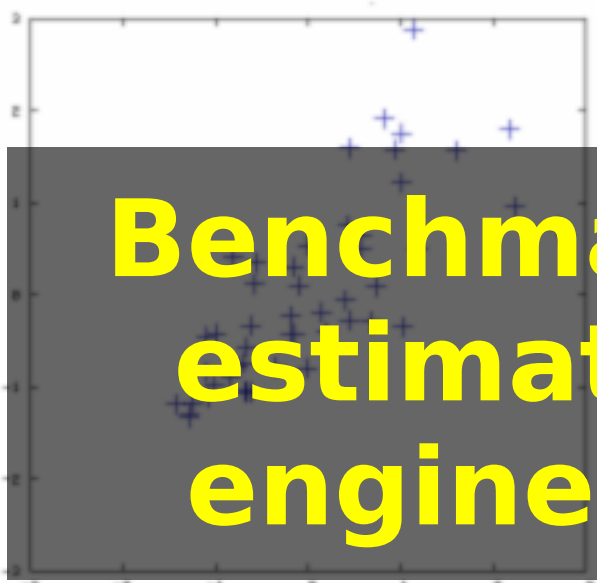
Benchmarking parameter estimation and reverse engineering strategies

Pedro Mendes, Diogo Camacho, Paola Vera-Licona, Reinhard Laubenbacher

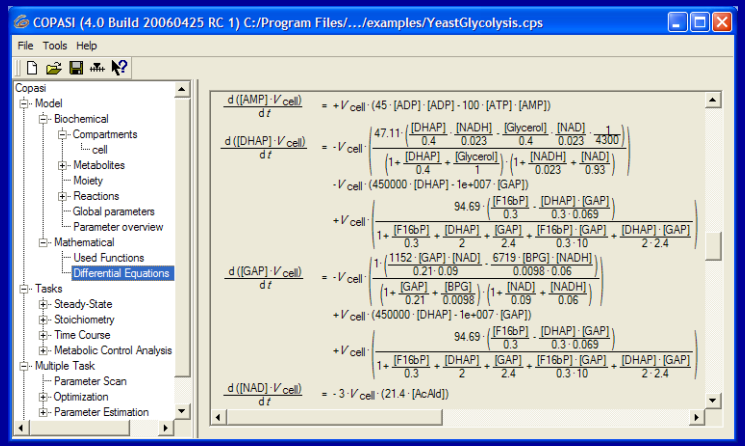
http://mendes.vbi.vt.edu

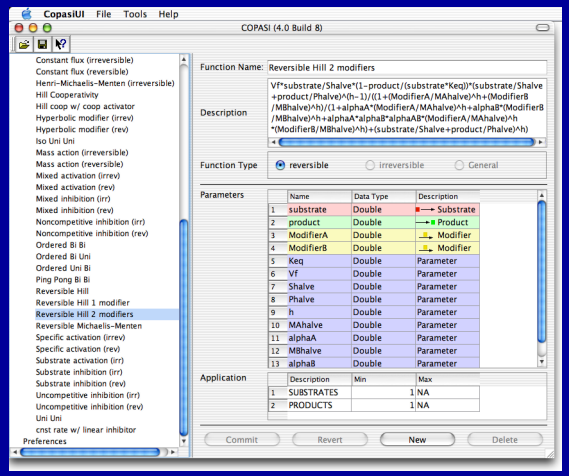


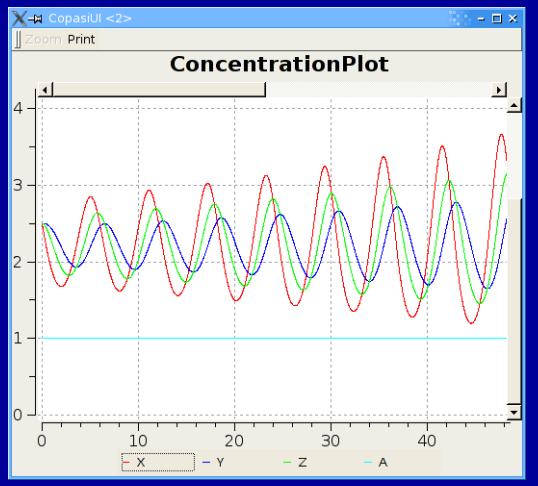
copasi

- ODE and stochastic simulation
- MCA, stability analysis, parameter scans
- Optimisation, fitting
- Sensitivity analysis
- GUI and command line versions
- Reads/writes SBML

- Mendes group
- Kummer group







http://www.copasi.org

BIOINFORMATICS ORIGINAL PAPER Vol. 22 no. 24 2006, pages 3067–3074
doi:10.1093/bioinformatics/btl485

Systems biology

COPASI—a COmplex PAthway SImulator

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ABSTRACT

Motivation: Simulation and modeling is becoming a standard approach to understand complex biochemical processes. Therefore, there is a big need for software tools that allow access to diverse simulation and modeling methods as well as support for the usage of these methods.
Results: Here, we present COPASI, a platform-independent and user-friendly biochemical simulator that offers several unique features. We discuss numerical issues with these features; in particular, the criteria to switch between stochastic and deterministic simulation methods, hybrid deterministic–stochastic methods, and the importance of random num-

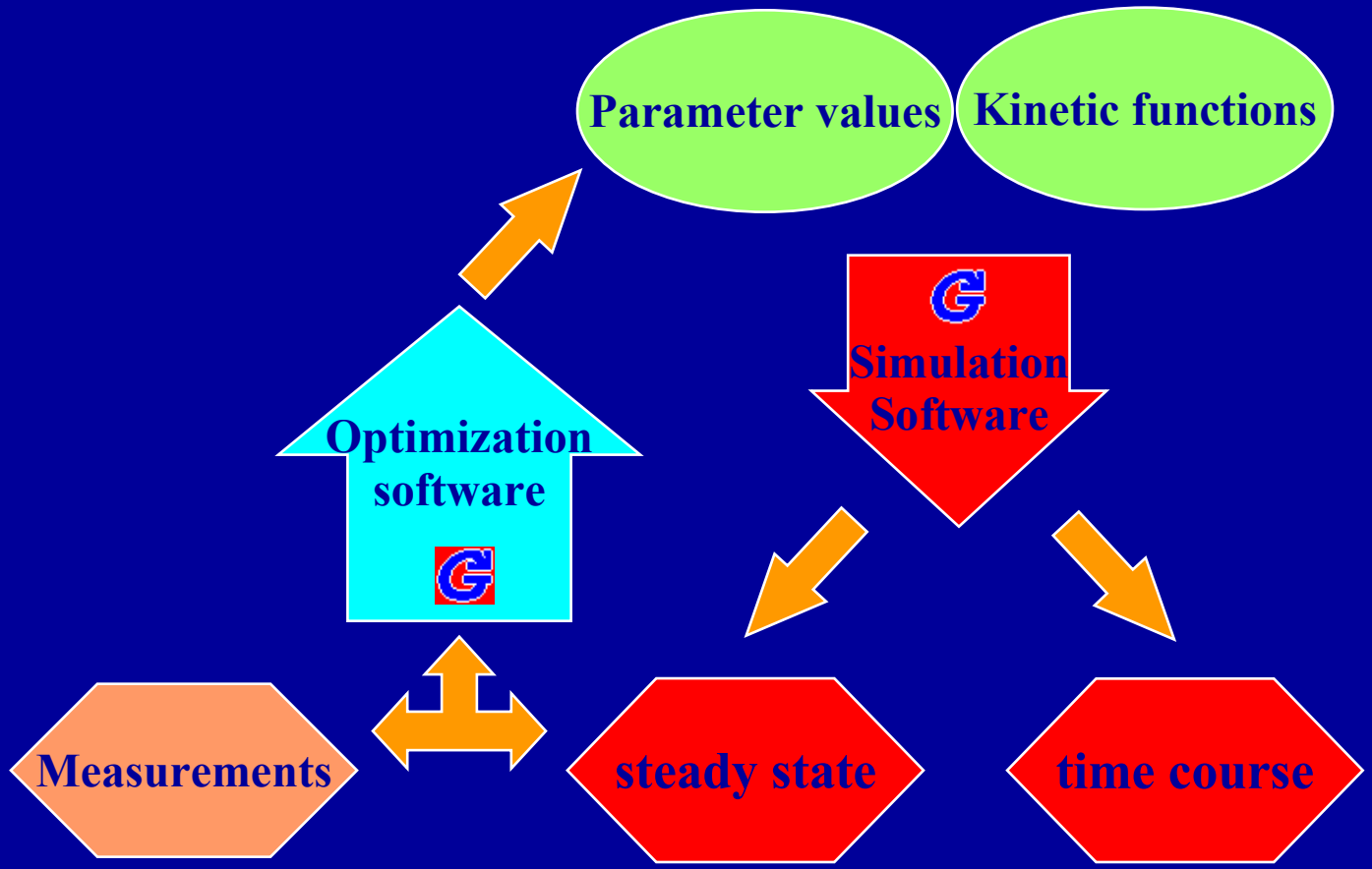
and flux analysis (Klamt *et al.*, 2003). However, some tools contain whole suites of functionalities, e.g. simulation, flux and control analysis (Tomita *et al.*, 1999; Sauro *et al.*, 2003; Meng *et al.*, 2004).

In order to improve the compatibility of these tools, markup languages such as SBML (Hucka *et al.*, 2003) and CellML (Lloyd *et al.*, 2004) were created to allow model exchange. Many tools are now able to read and write models in these file formats.

Here we present a new program—COPASI (COmplex PAthway SImulator)—which combines all of the above standards and some unique methods for the simulation and analysis of biochemical

Hoops *et al.* (2006) *Bioinformatics* **22**, 3067-3074

Parameter estimation



Optimization methods

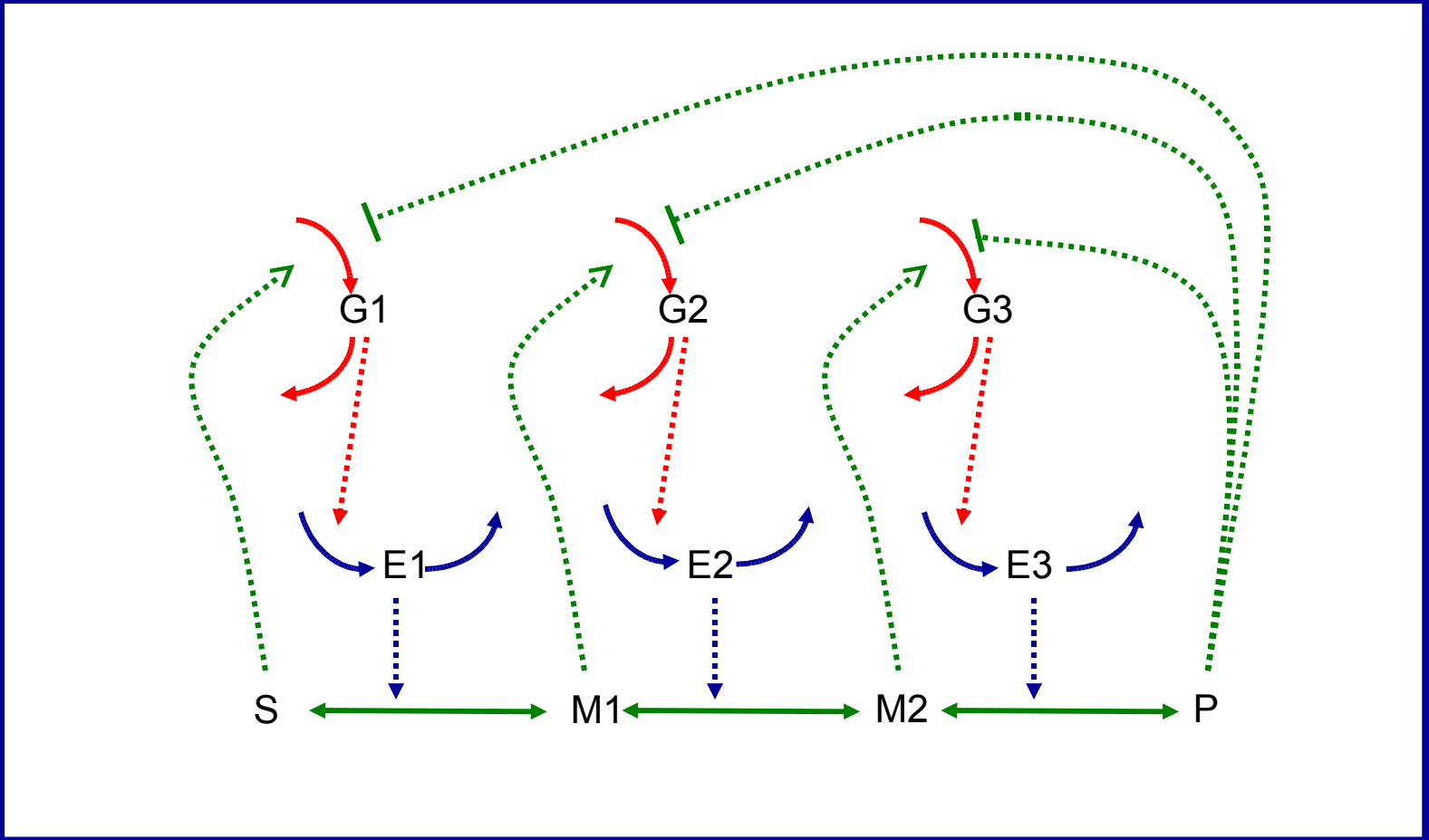
Based on derivatives:

- L-BFGS-B
- Levenberg-Marquardt
- Steepest descent
- Truncated Newton
- NL2SOL
- Derivative Tensor method

Direct search:

- Hooke & Jeeves
- Nelder & Mead (simplex)
- Multistart
- Genetic algorithm
- Evolutionary programming
- Simulated annealing
- Particle swarm
- Random search

3-step pathway with gene expression



3-step model

- Total of 39 parameters, 3 are equilibrium constants (independent of the biology),
- Total of 36 adjustable parameters to fit the model to the time course data
- Two classes of parameters:
  - Hill coefficients: allowed to vary [0.1:10]
  - All others: allowed to vary [10^{-12}:10^{6}]

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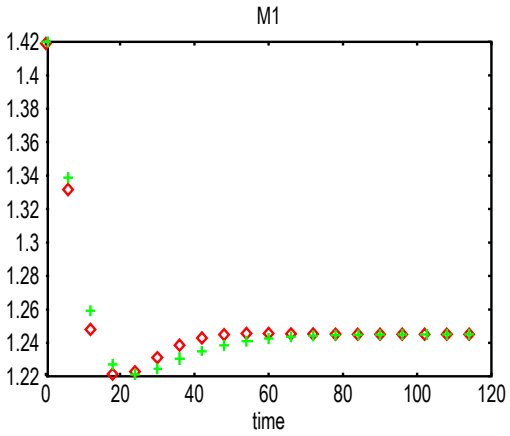
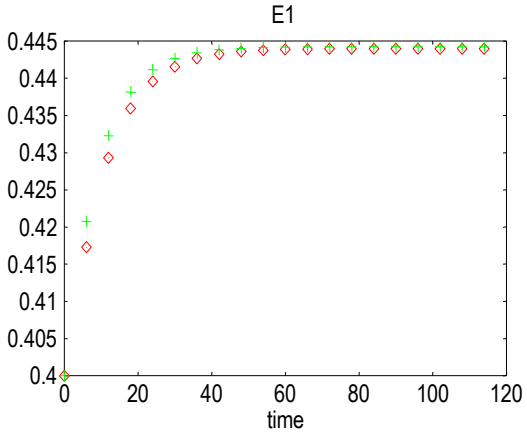
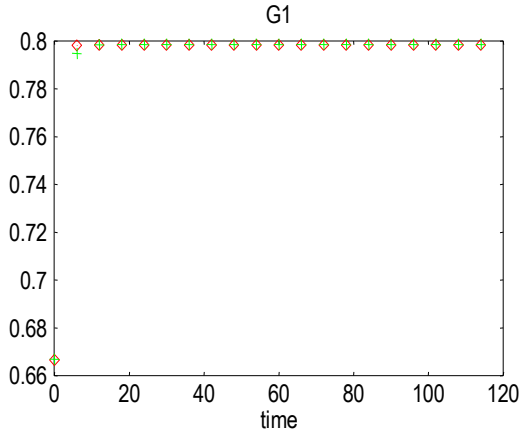
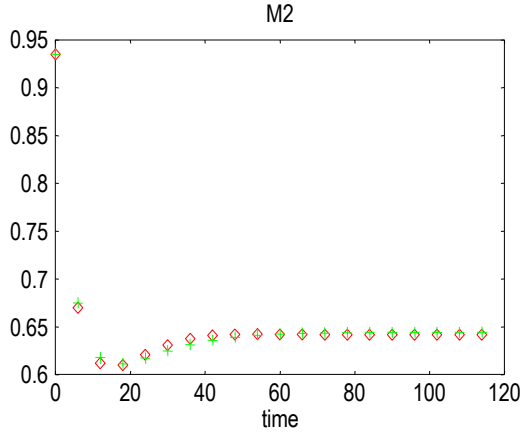
3-step model – results

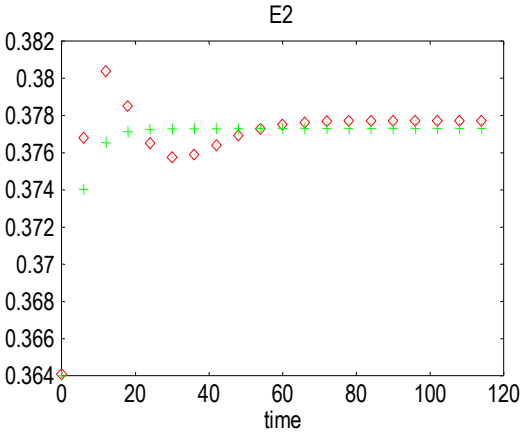
- Gradient methods could not converge to the solution from an arbitrary starting point
- Evolutionary programming converged to a small sum of squares…
- … which could be further improved with the Hooke and Jeeves method…
- …and finally with Levenberg-Marquardt
- The final solution replicates the original dynamics rather well
- Estimated parameter values sometimes deviate by 10x

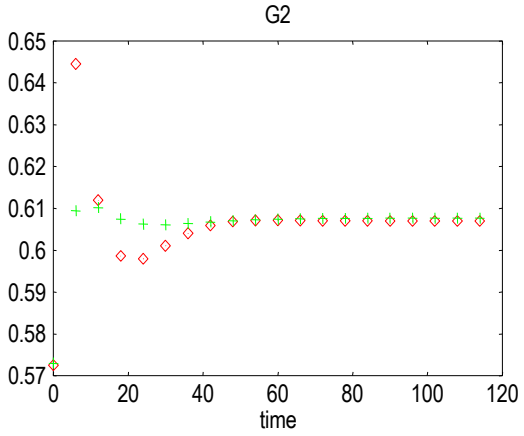


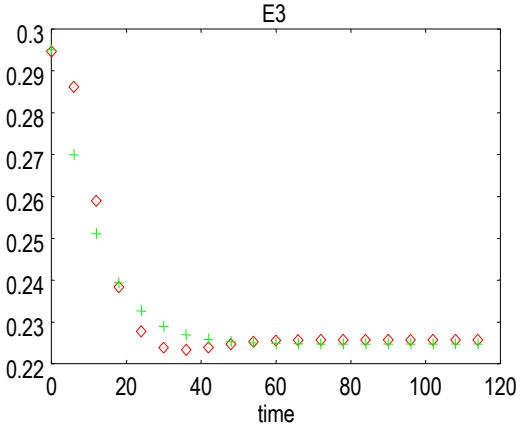


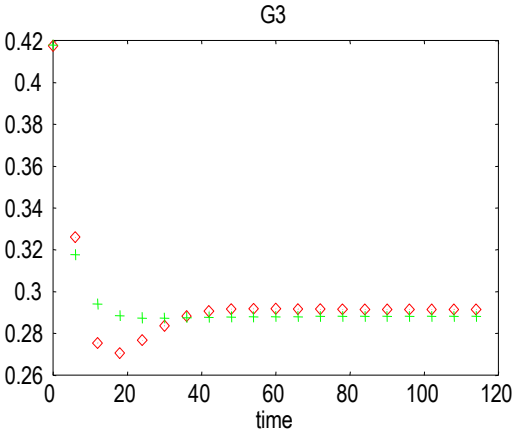












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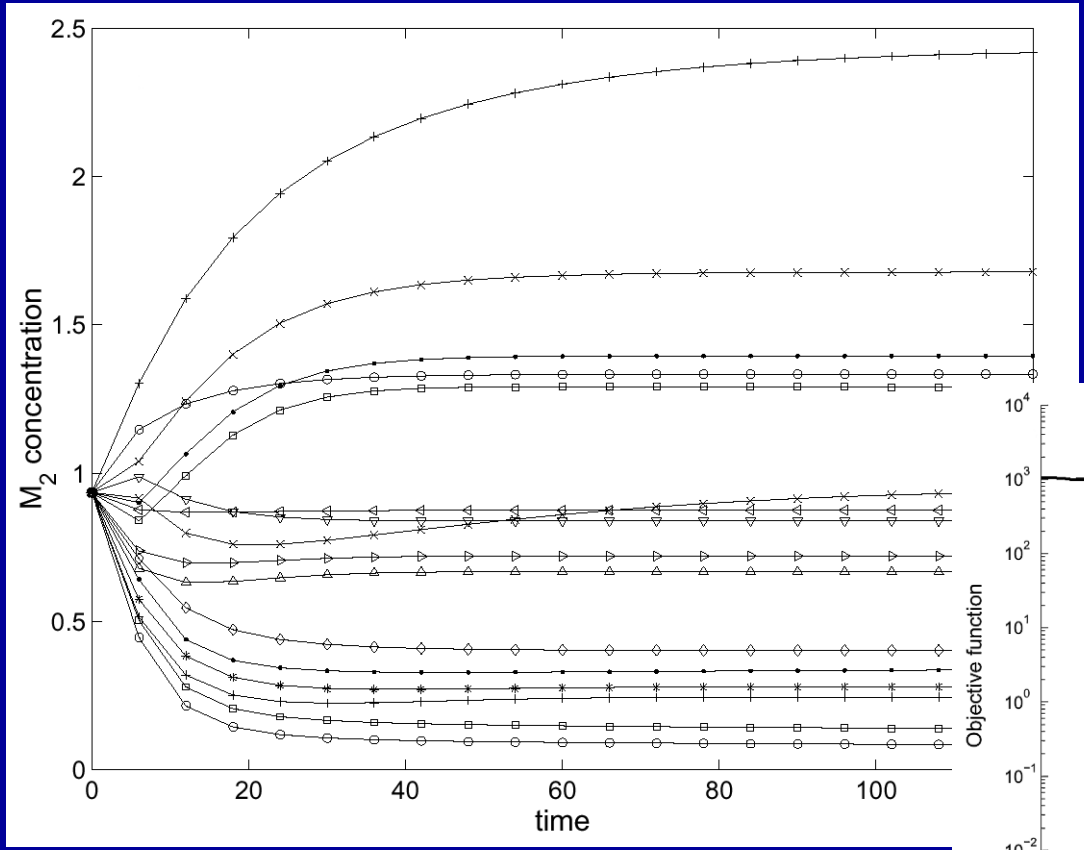
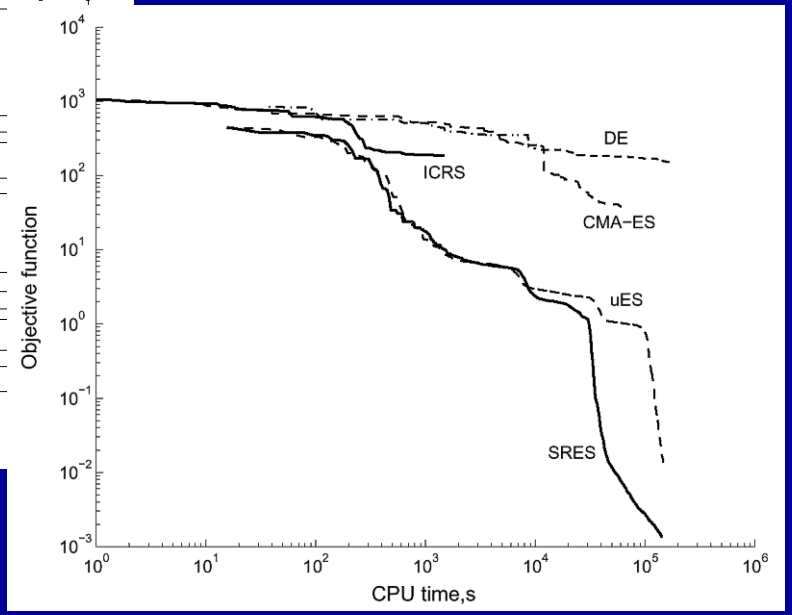
Results, 2nd attempt…



- 16 trajectories
- New minimization algorithms
- Worst parameter estimate off only by 16%



Moles, C.G., Mendes, P. and Banga, J.R. (2003) Parameter estimation in biochemical pathways: a comparison of global optimization methods. *Genome Research* **13**, 2467-74.

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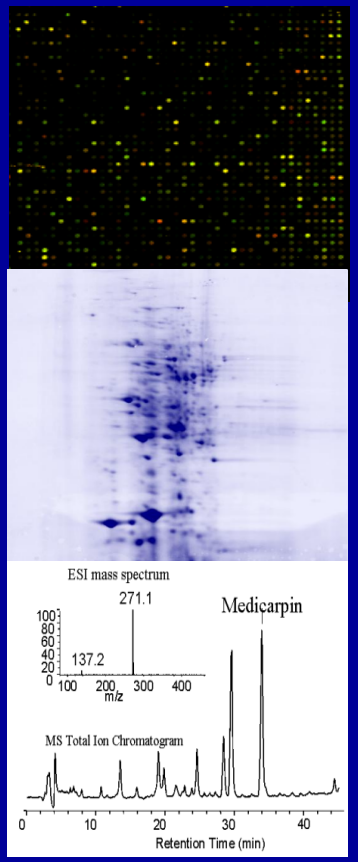
Issues with parameter estimation

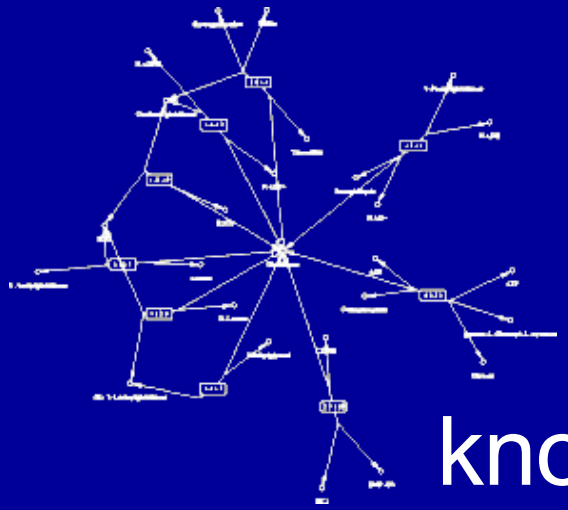
- Thousands of variables, will require extensive parallelization / distributed computing
- Functions (rhs) must be known *a priori*
- The objective function has several minima
- Data sets will often be under-determined

Top-down modeling
Reverse engineering



data

ATP	5.01
NADH	1.73
NADPH	6.21
ADP	9.91
Orthophosphate	6.7
CoA	4.15
Pyrophosphate	8.57
NH3	9.46
S-Adenosyl-L-methionine	6.24
AMP	8.49
S-Adenosyl-L-homocysteine	7.54
Pyruvate	1.22
Acetyl-CoA	4.53
L-Glutamate	1.17
2-Oxoglutarate	0.21
UDPglucose	4.83
D-Glucose	3.77
Acetate	3.4
GDP	9.55
Oxaloacetate	9.61
Glycine	4.53
L-Alanine	0.81
Succinate	0.62
UDP-N-acetyl-D-glucosamine	3.65
GTP	9
L-Lysine	6.79
Glyoxylate	0.04
L-Aspartate	6.43
Glutathione	5.52
UDP-D-galactose	0.92
Formate	7.25
L-Arginine	0.52
L-Glutamine	2.65
L-Serine	3.46
Formaldehyde	0.43
Thiamin diphosphate	9.35
Alcohol	7.61
Ascorbate	9
L-Methionine	8.8
Phosphoenolpyruvate	3.85
L-Ornithine	7.49
L-Tryptophan	6.35
L-Phenylalanine	5.07
L-Tyrosine	2.01
Malonyl-CoA	7.52
Acetaldehyde	5.28
D-Fructose 6-phosphate	6.84
Sucrose	1.29



knowledge

$$\dot{A} = \frac{V_1^f \frac{S}{K_{1S}} - V_1^r \frac{A}{K_{1A}}}{1 + \frac{S}{K_{1S}} + \frac{A}{K_{1A}}} - \frac{\left(V_2^f \frac{A}{K_{2A}}\right)\left(1 - \frac{B}{S \cdot K_{2eq}}\right)\left(\frac{A}{K_{2A}} + \frac{B}{K_{2B}}\right)^{h-1}}{\left(\frac{A}{K_{2A}} + \frac{B}{K_{2B}}\right)^{h} + \frac{1 + \left(\frac{C}{K_{2C}}\right)^{h}}{1 + \alpha\left(\frac{C}{K_{2C}}\right)^{h}}}$$

$$\dot{B} = \frac{\left(V_2^f \frac{A}{K_{2A}}\right)\left(1 - \frac{B}{S \cdot K_{2eq}}\right)\left(\frac{A}{K_{2A}} + \frac{B}{K_{2B}}\right)^{h-1}}{\left(\frac{A}{K_{2A}} + \frac{B}{K_{2B}}\right)^{h} + \frac{1 + \left(\frac{C}{K_{2C}}\right)^{h}}{1 + \alpha\left(\frac{C}{K_{2C}}\right)^{h}}} - \frac{V_3^f \frac{B}{K_{3B}} - V_3^r \frac{C}{K_{3C}}}{1 + \frac{B}{K_{3B}} + \frac{C}{K_{3C}}}$$

$$\dot{C} = \frac{V_3^f \frac{B}{K_{3B}} - V_3^r \frac{C}{K_{3C}}}{1 + \frac{B}{K_{3B}} + \frac{C}{K_{3C}}} - \frac{V_4^f \frac{C}{K_{4C}} - V_4^r \frac{P}{K_{4P}}}{1 + \frac{C}{K_{4C}} + \frac{P}{K_{4P}}}$$

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The DREAM Project

Assessing the Accuracy of Reverse Engineering Methods

AcademyeBriefings

http://www.nyas.org/ebriefreps/main.asp?intSubsectionID=3962

- Led by Andrea Califano (Columbia Univ.) and Gustavo Stolovitzky (IBM)
- Data and techniques to understand how well reverse engineering methods can infer the underlying biochemical networks in the cell.
- No biological gold standard available:
  - Biological data/networks (convincing but fuzzy)
  - Engineered networks (biological, yet known)
  - In silico models (full control, skepticism)
- Inspired by CASP, is expected to have a similar effect on systems biology

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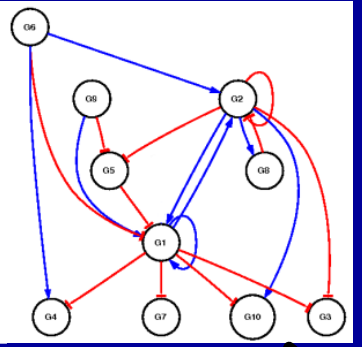
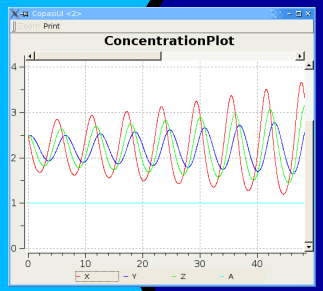
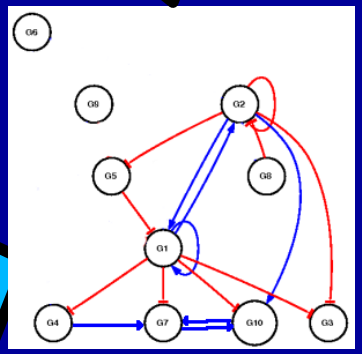
In silico biochemical networks for algorithm comparison

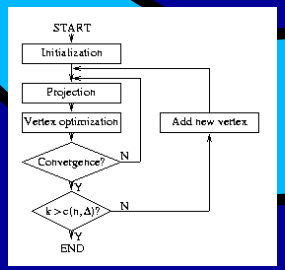
$$v_s = V \cdot \prod_j \left(\frac{Ki_j^{n_j}}{(I_j^{n_j} + Ki_j^{n_j})} \right) \cdot \prod_k \left(1 + \frac{A_k^{n_k}}{(A_k^{n_k} + Ka_k^{n_k})} \right)$$

$$v_s = V \cdot \prod_j \left(\frac{Ki_j^{n_j}}{(I_j^{n_j} + Ki_j^{n_j})} \right) \cdot \prod_k \left(1 + \frac{A_k^{n_k}}{(A_k^{n_k} + Ka_k^{n_k})} \right)$$









- Different experiments can be simulated
- Network is known exactly thus provides accurate metrics
- Performance of algorithms against noise can be measured
- Network details can be kept secret => blind tests

- Can these networks be made realistic enough to be relevant?

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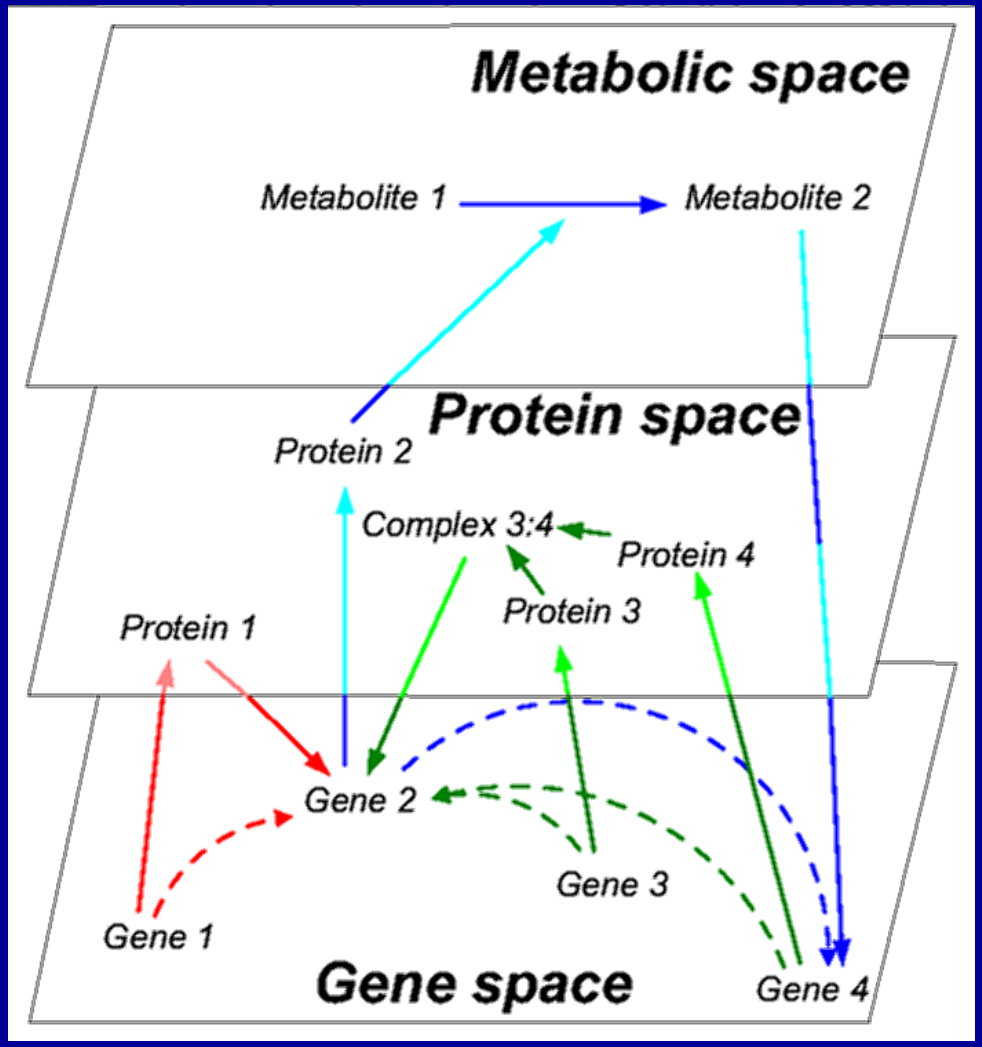
Biochemical networks



Brazhnik, P., de la Fuente, A. & Mendes, P. (2002) *Trends in Biotechnology* **20**, 467-472.

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BIOINFORMATICS

Vol. 19 Suppl. 2 2003, pages ii122–ii129
DOI: 10.1093/bioinformatics/btg1069

Artificial gene networks for objective comparison of analysis algorithms

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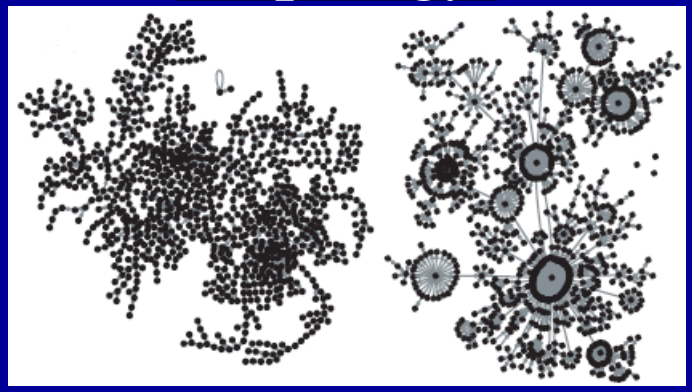
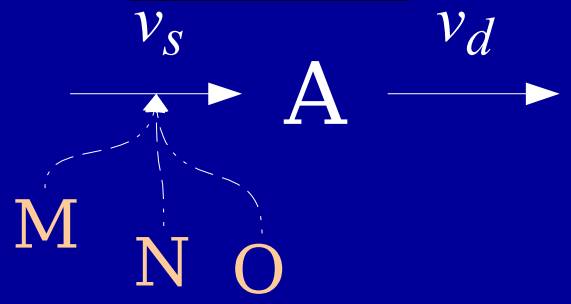
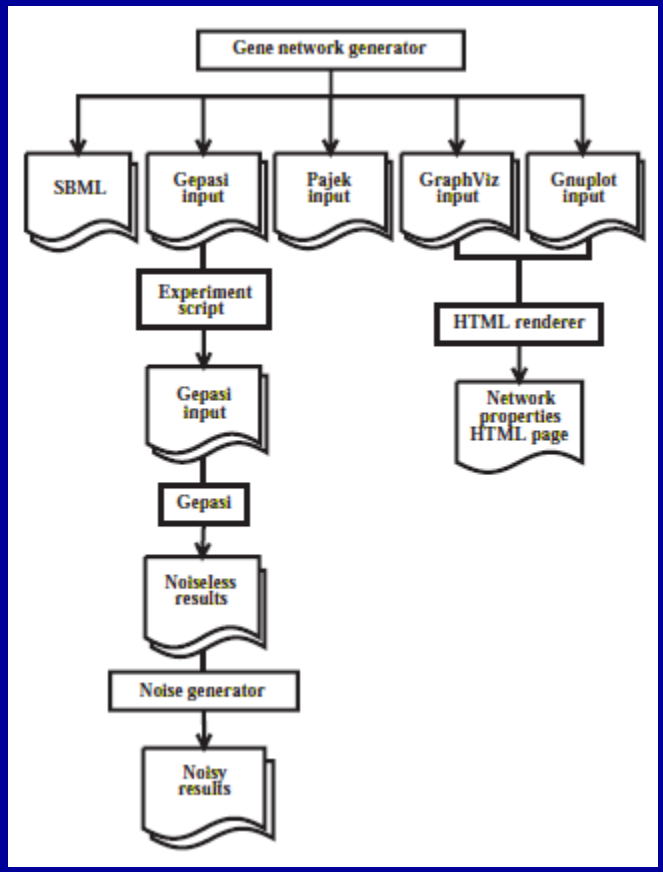
Topology



Kinetics



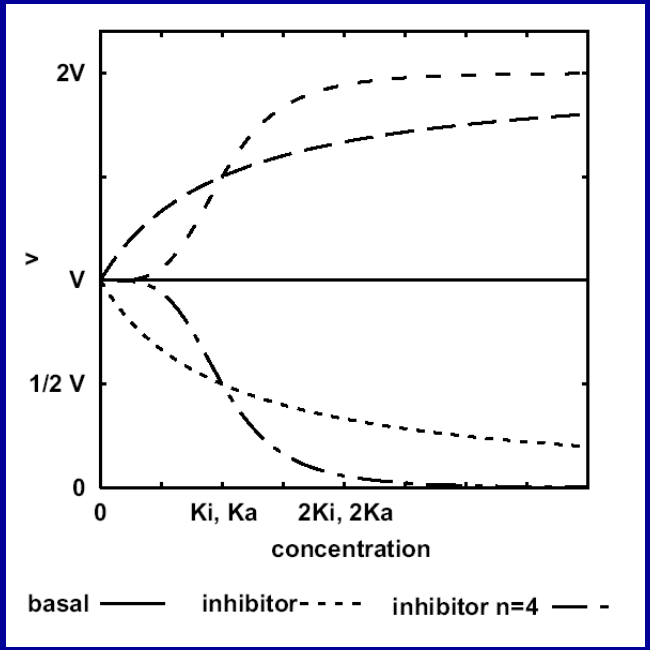


Kinetics of transcription

- Rate is saturable due to finite number of translation machinery complexes
- Rate is inhibited by a number of inhibitor genes
- Rate is activated by a number of activator genes
- Inhibition and activation can display cooperativity
- Inhibitors and activators act independently
- Rate is assumed insensitive to nucleotide concentrations

Kinetics for AGN

$$v_s = V \cdot \prod_j \left(\frac{Ki_j^{n_j}}{(I_j^{n_j} + Ki_j^{n_j})} \right) \cdot \prod_k \left(1 + \frac{A_k^{n_k}}{(A_k^{n_k} + Ka_k^{n_k})} \right)$$



- V limiting rate
- Ki inhibition constants
- ni Hill coefficient for inhibitors
- Ka activation constants
- na Hill coefficient for activators

$$v_d = k_d \cdot A$$

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Adding noise to simulated data

- It is often important to study the effect of different sources of “noise” on measurements
- Additive noise: due to measurement noise; added to simulated data at the end
- Intrinsic noise: due to time-dependent processes; injected at all points in the network continuously
- Biological variance: due to differences in cells, individuals, and cultures; added to parameters before the simulation

Evaluation of Statistical Methods in Microarray Differential Expression Analysis

1) Welch t test
2) ANOVA fixed model for each gene
3) ANOVA mixed model for each gene
4) ANOVA mixed model for whole experiment

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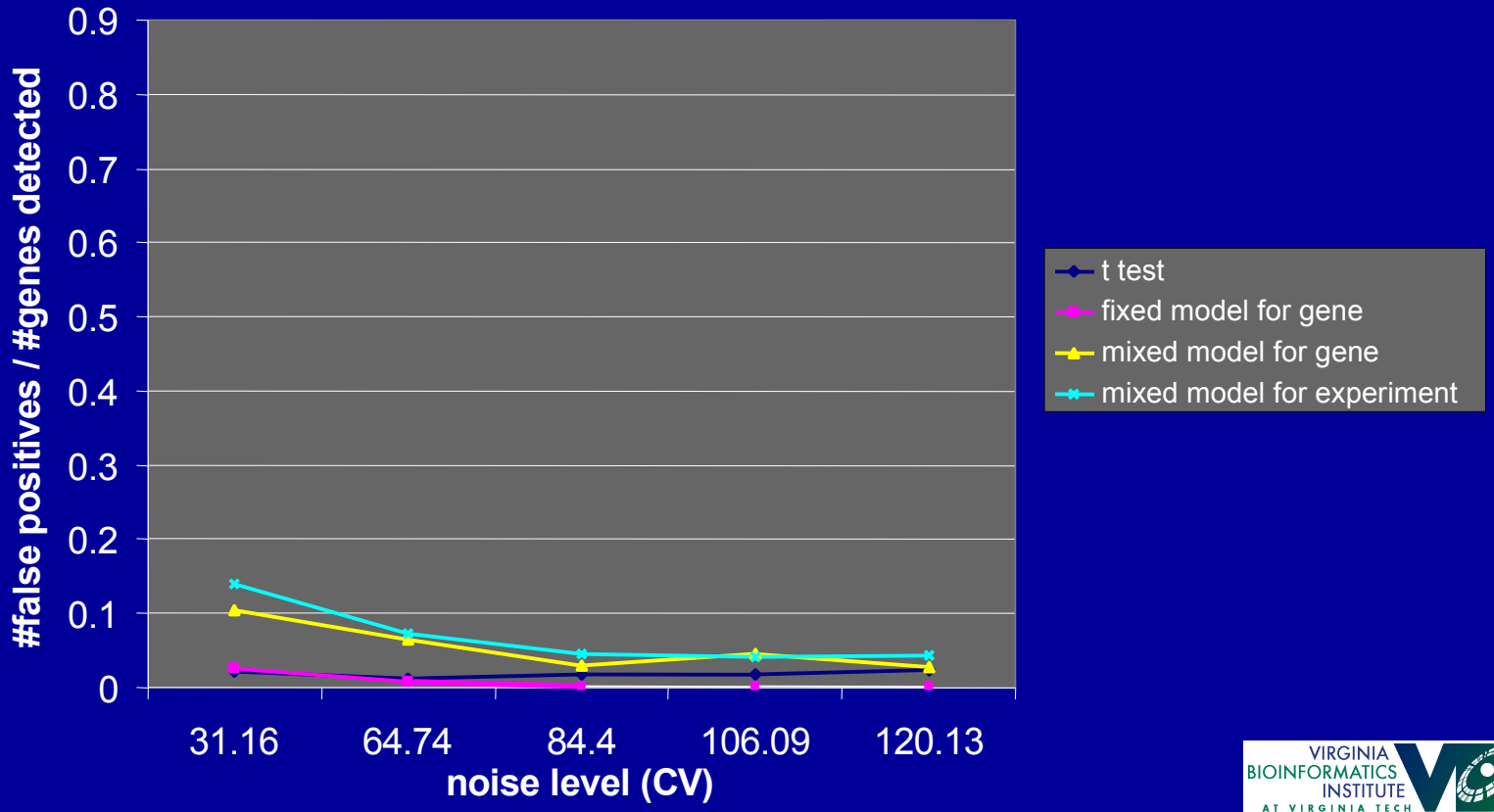
Comparison: false positives

(*P* values adjusted to constant FDR of 5%)



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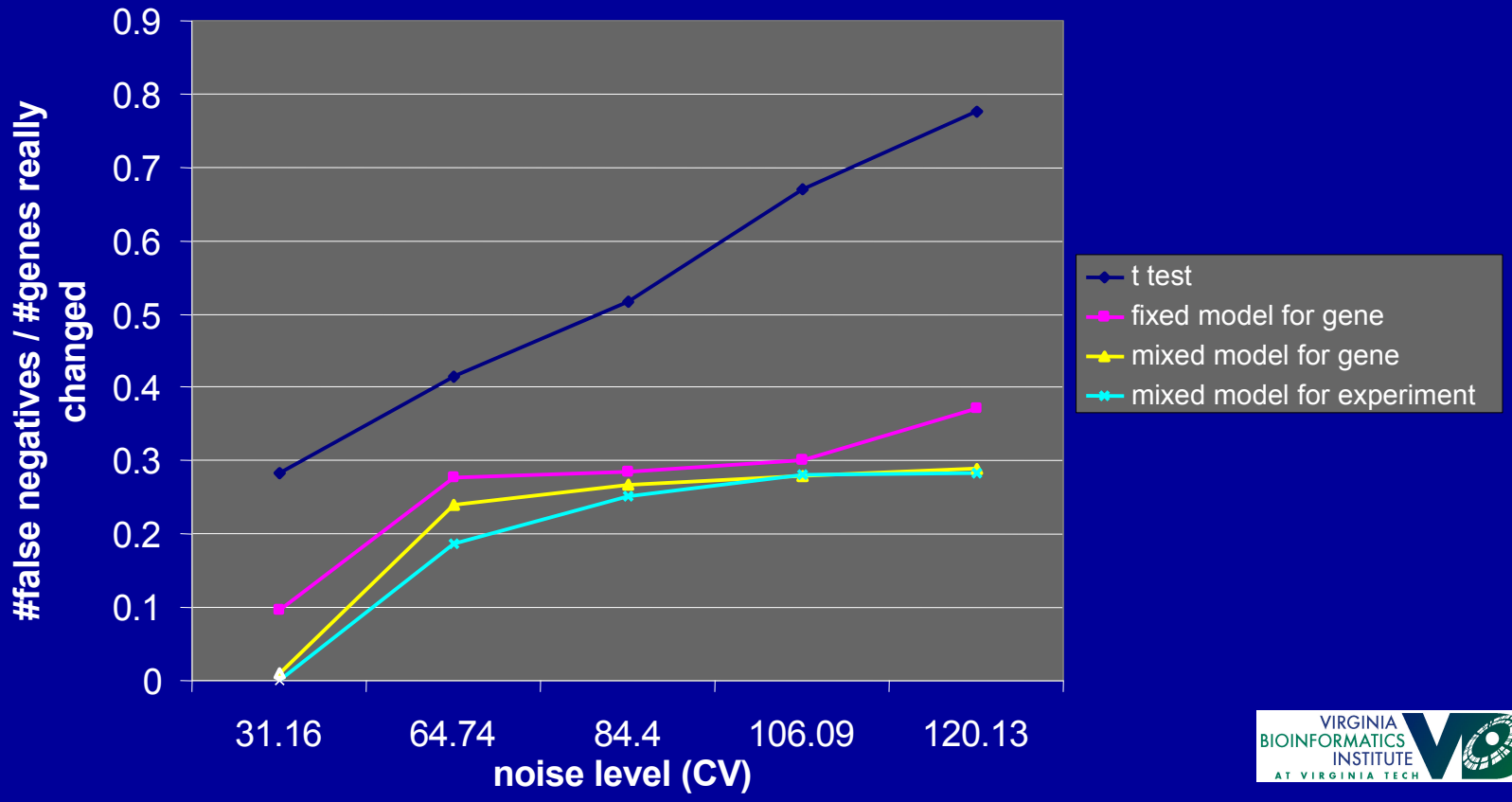
Comparison: false negatives

(*P* values adjusted to constant FDR of 5%)



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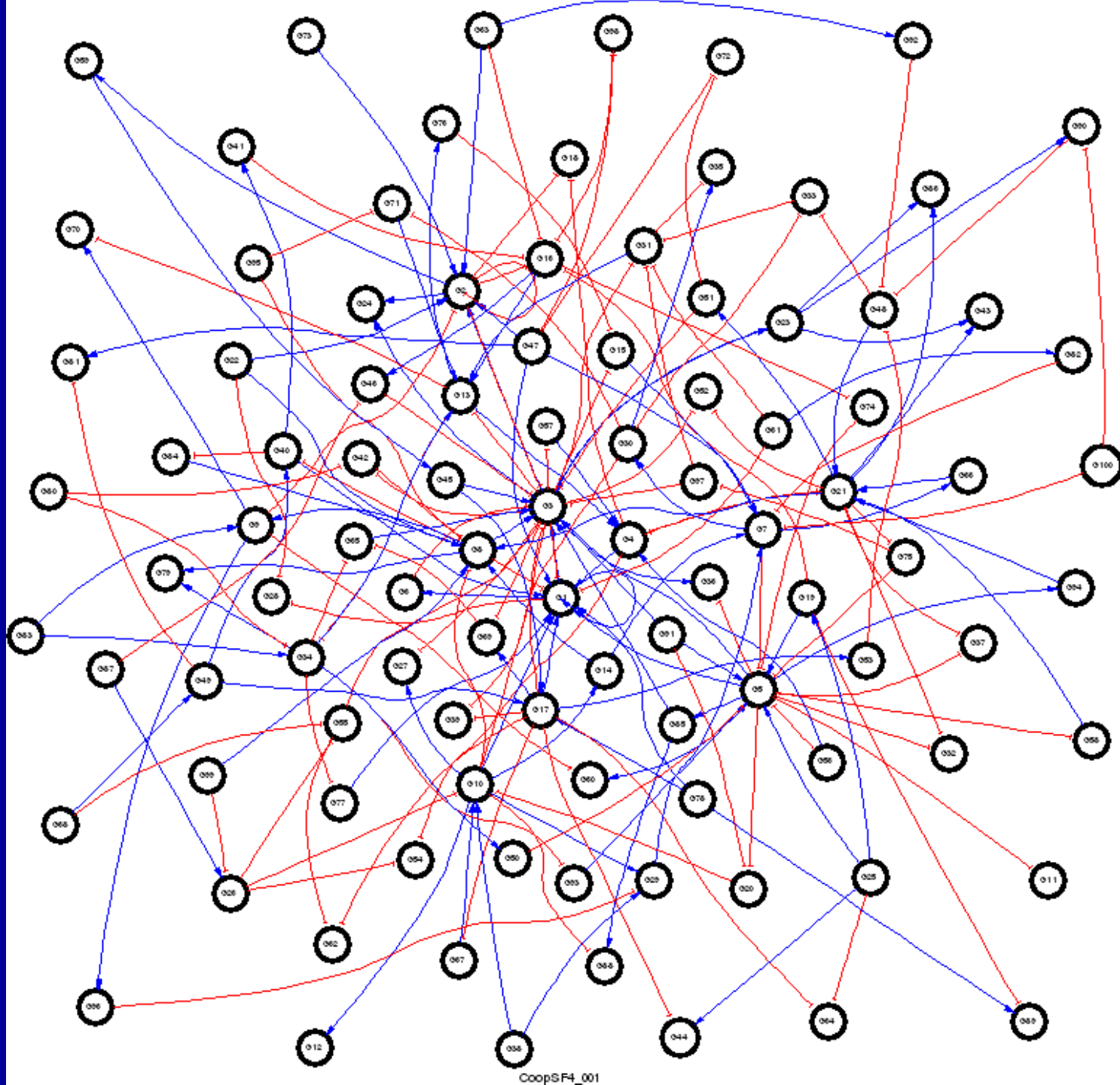
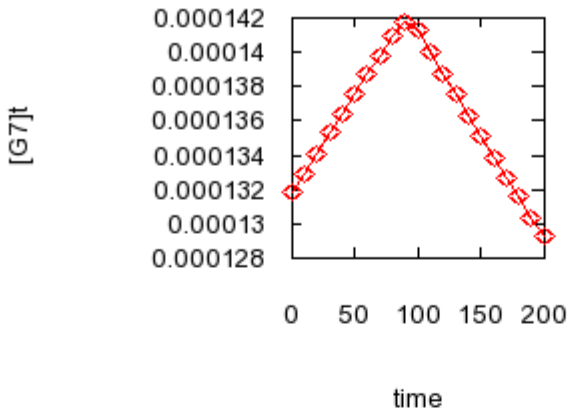
Effect of kinetics

- Original networks had similar kinetics for all nodes:
  - Similar time scales
  - Similar ranges
- For a single network (CoopSF41), parameters were adjusted to obtain "interesting" behavior
  - Several time scales
  - Widely different ranges
  - Strongly nonlinear behavior

CoopSF41:

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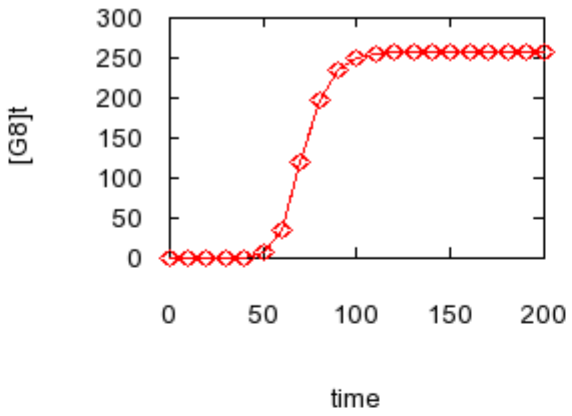
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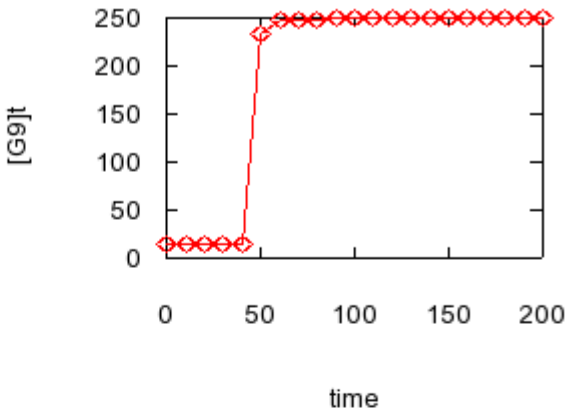
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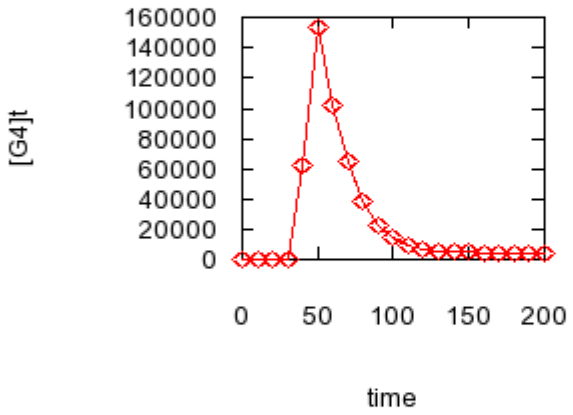
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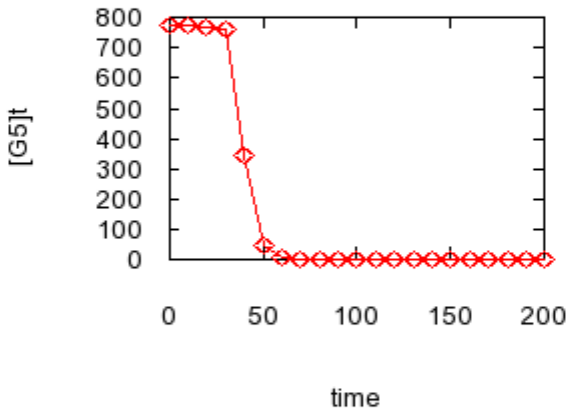
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800
700
600
500
400
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0
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50
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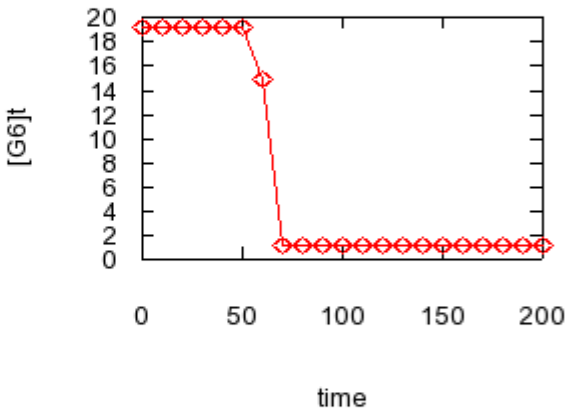
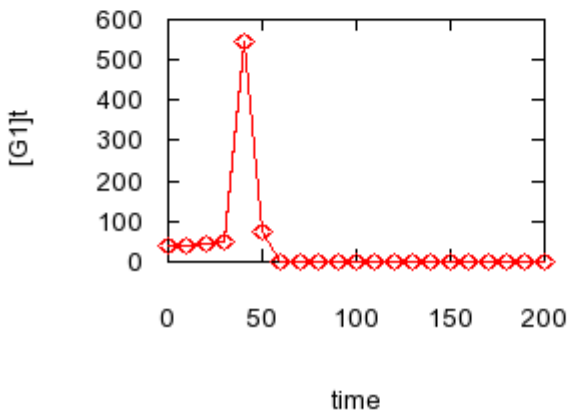
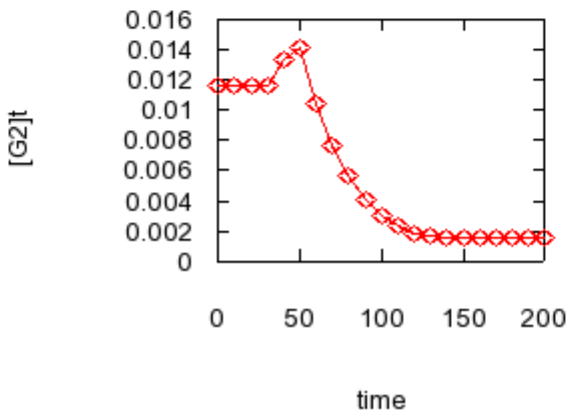
150
200
time


[G6]t
20
18
16
14
12
10
8
6
4
2
0
0
50
100
150
200
time


[G1]t
600
500
400
300
200
100
0
0
50
100
150
200
time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0.004
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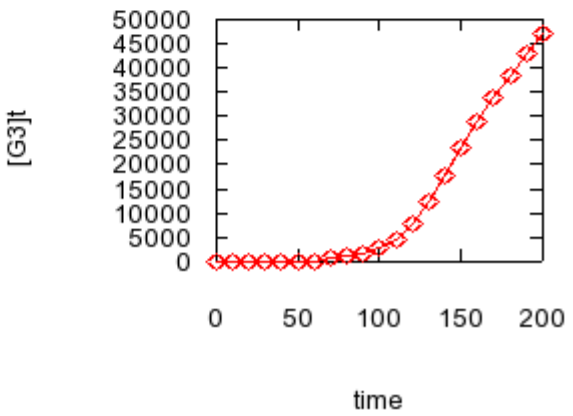
[G3]t
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40000
35000
30000
25000
20000
15000
10000
5000
0
0
50
100
150
200
time

Metrics

Confusion matrix

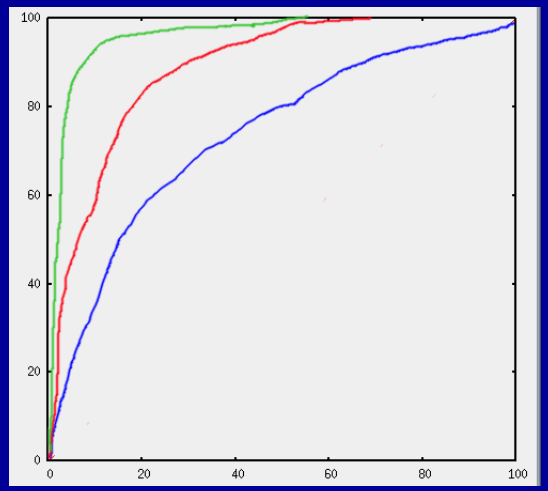
- Measures biases on inference of connections

predicted

real	+	0	-	
+	7	2	1	70.00%
0	3	10	2	66.67%
-	1	2	8	72.73%
	63.64%	71.43%	72.73%	69.44%

ROC curves

- Effect of algorithm tuning parameters on specificity and sensitivity



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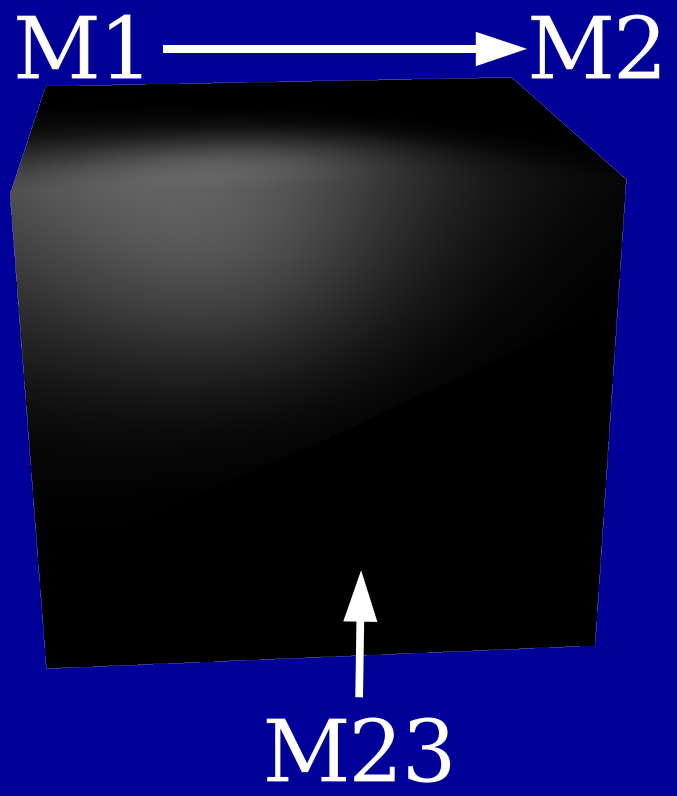
How about the rest of biochemistry ?

- To approach what happens in real cells, we need to include metabolism and signaling
- Metabolism is a very rigid network
  - A few cofactors link many reactions (ATP, NADP, CoA)
  - There are branches almost at every metabolite
- Signaling transduction is very flexible
  - Large crosstalk between pathways
  - Integration of signals and combinatorial responses

The Claytor Network

- Transcription
- Translation
- Metabolism
- Signal transduction
- 20 genes
- 23 protein forms
- 16 metabolites
- 3 transcription factors
- 2 receptors



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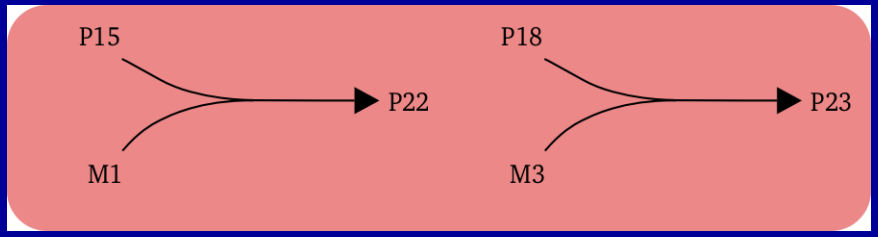
Protein synthesis and signaling

G1 → P1
G2 → P2
G3 → P3
G4 → P4
G5 → P5
G6 → P6
G7 → P7
G8 → P8
G9 → P9
G10 → P10
G11 → P11
G12 → P12
G13 → P13
G14 → P14
G15 → P15
G16 → P16
G17 → P17
G18 → P18
G19 → P19
G20 → P20



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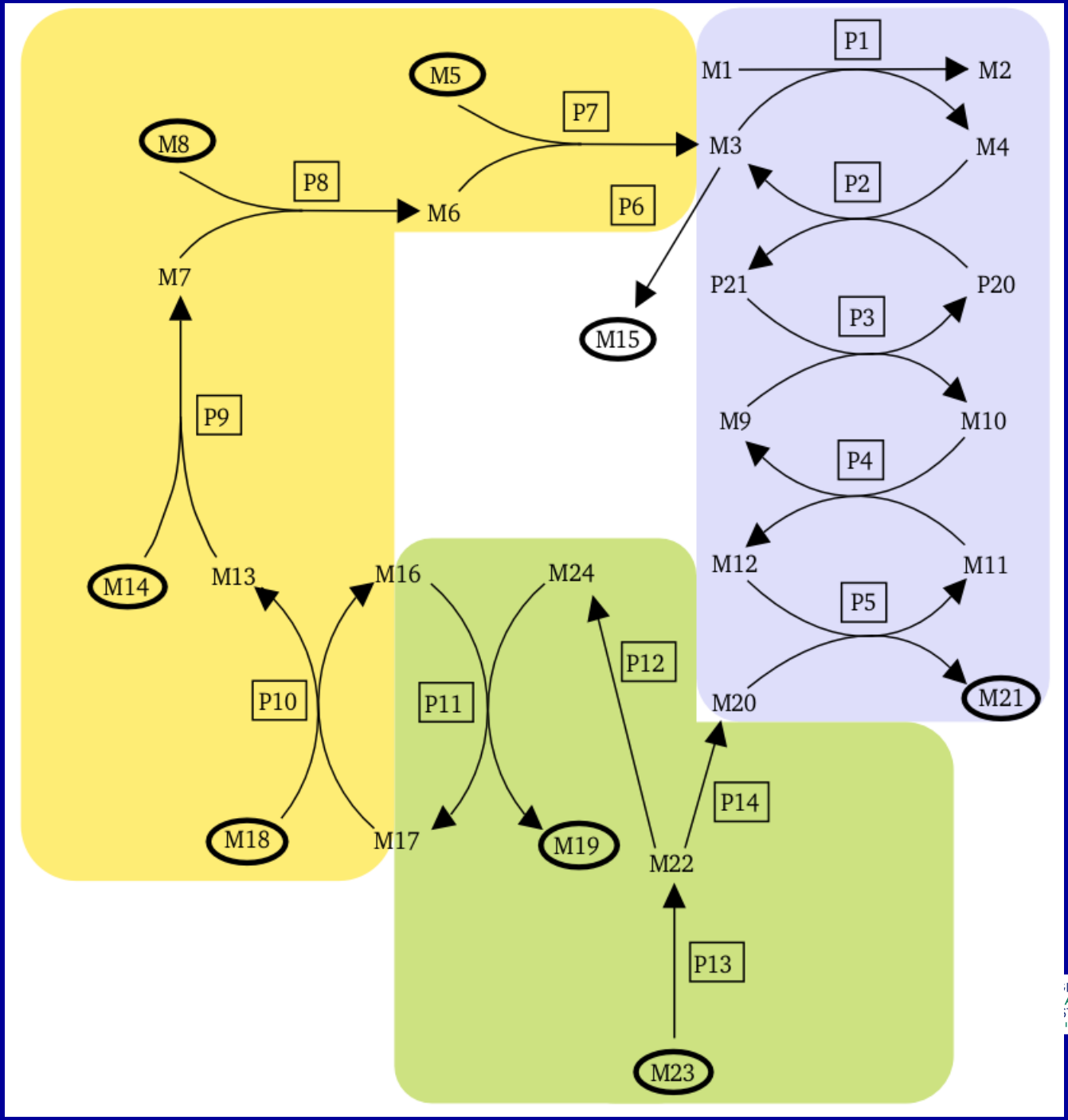
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M24
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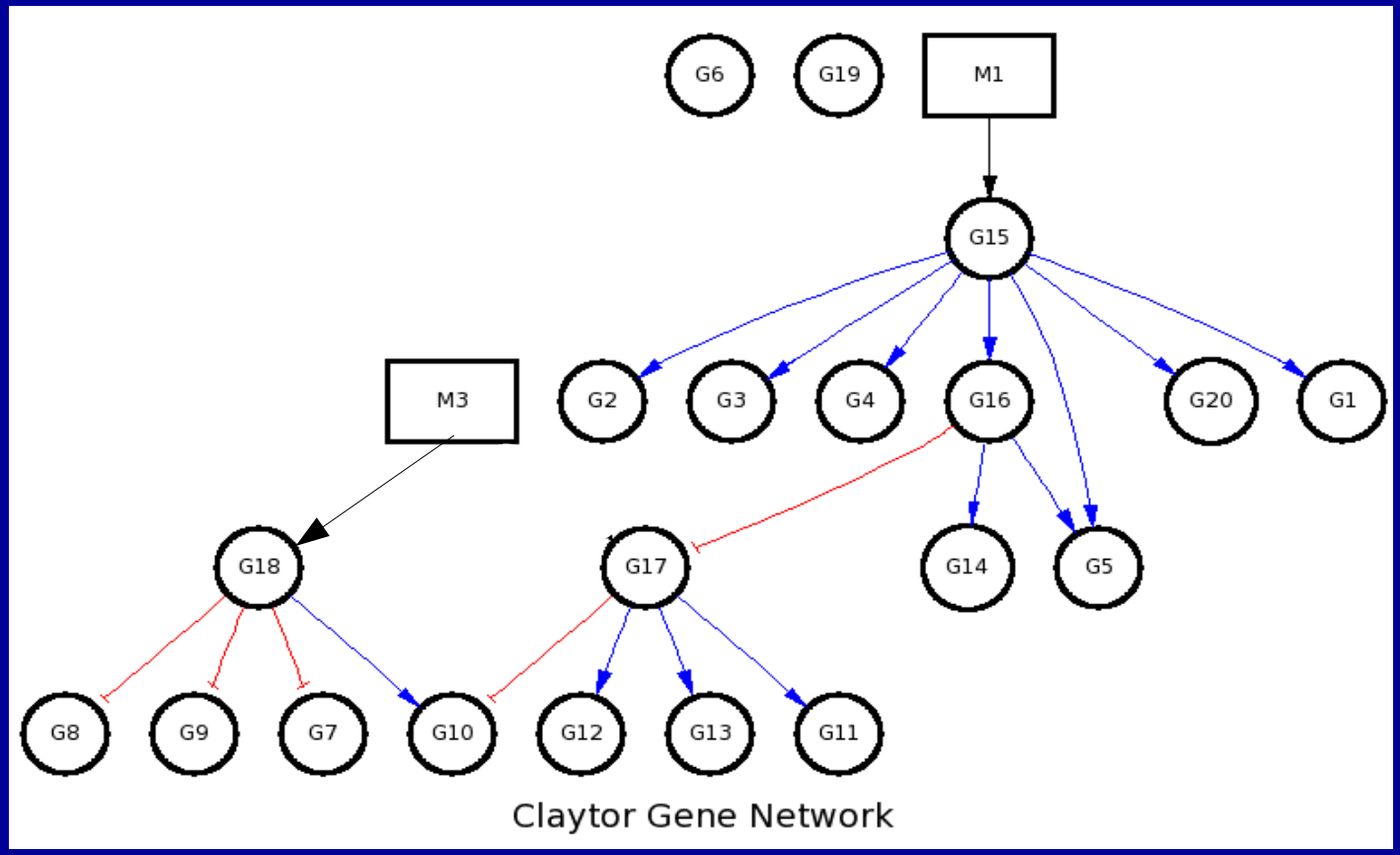
Genetic regulation



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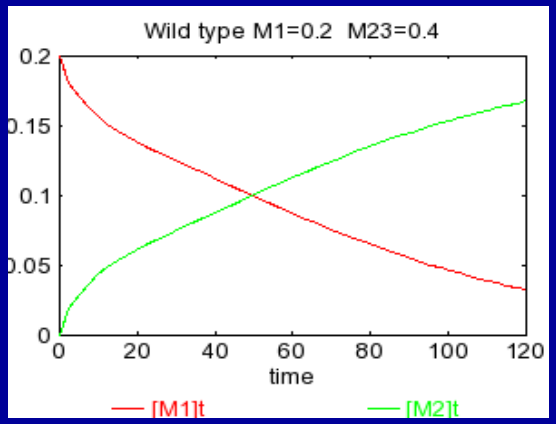
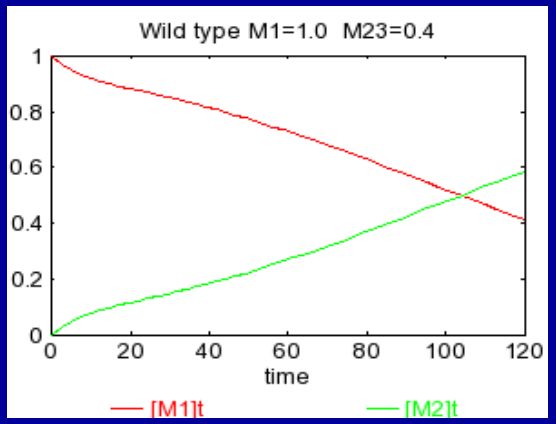
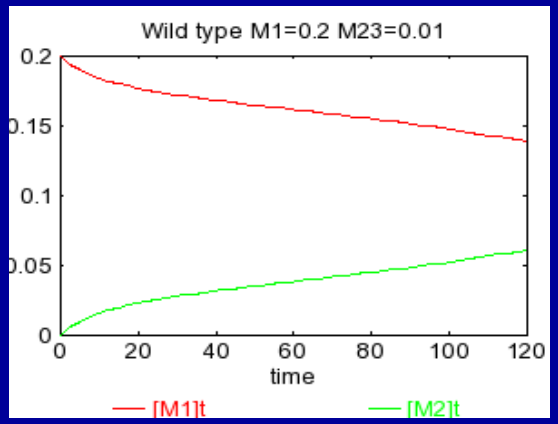
Gene knock-out experiments

STRAINS

- Wild type
- ΔG1
- ΔG6
- ΔG15
- ΔG16
- ΔG17
- ΔG18
- ΔG19







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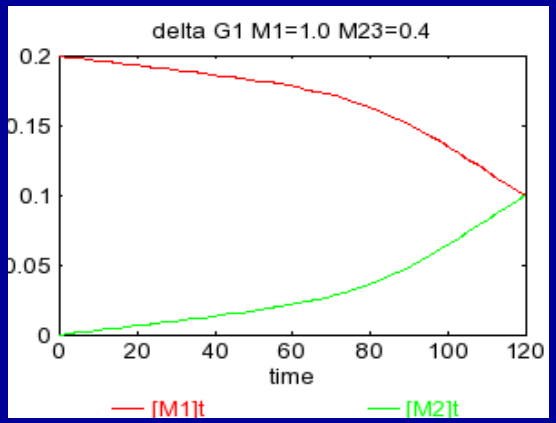

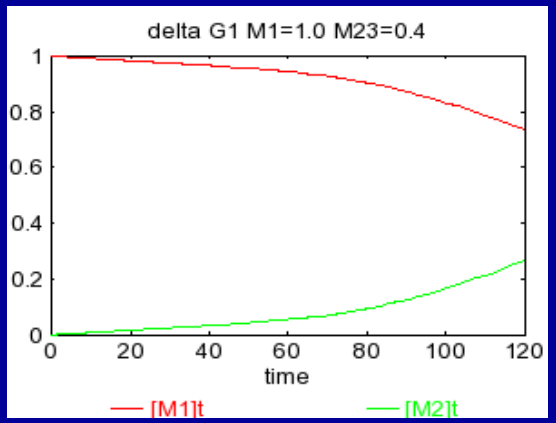

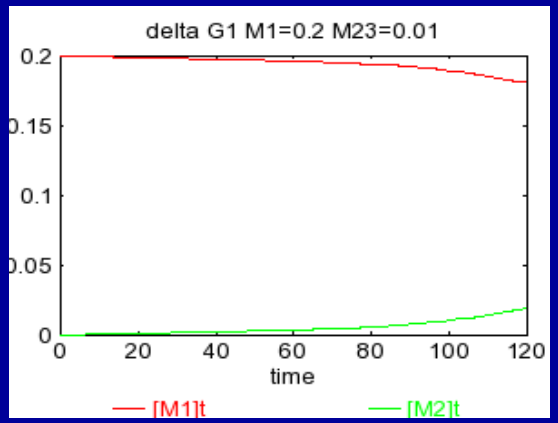

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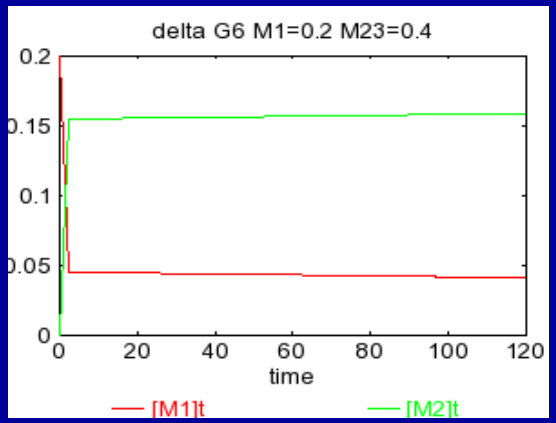

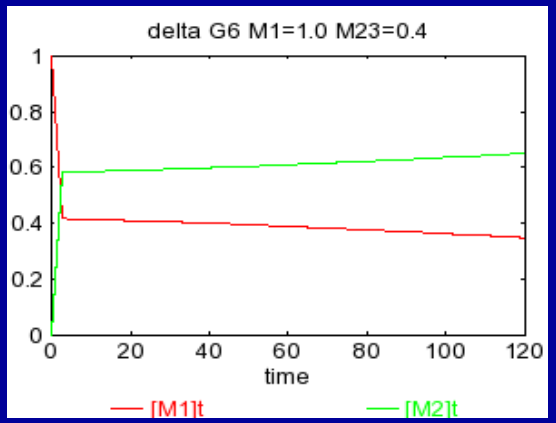

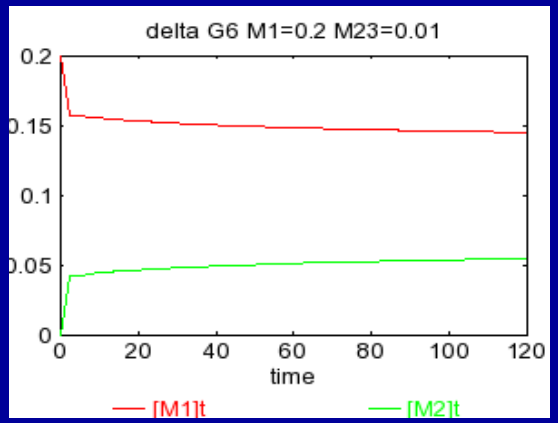

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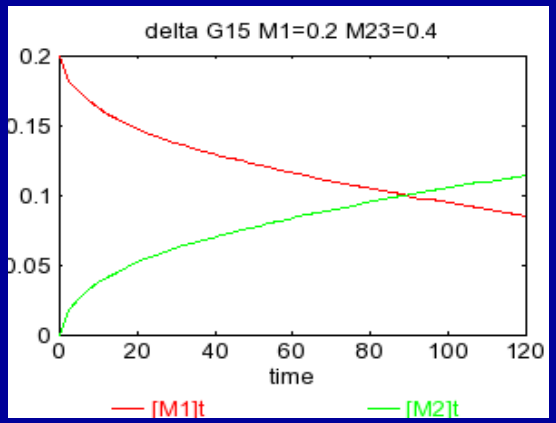

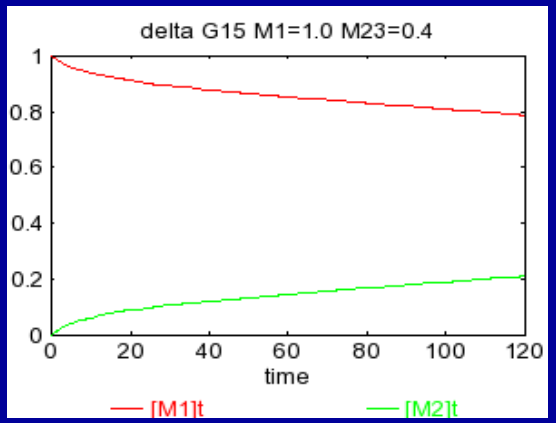

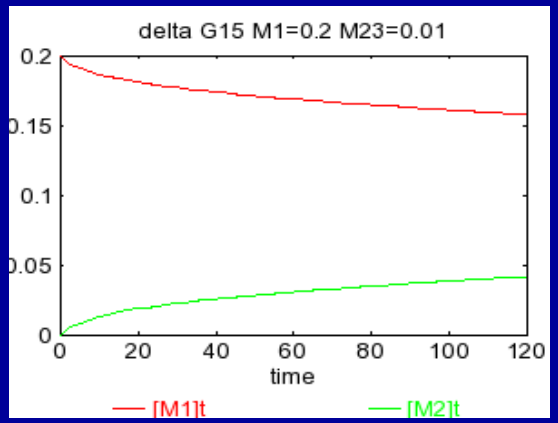

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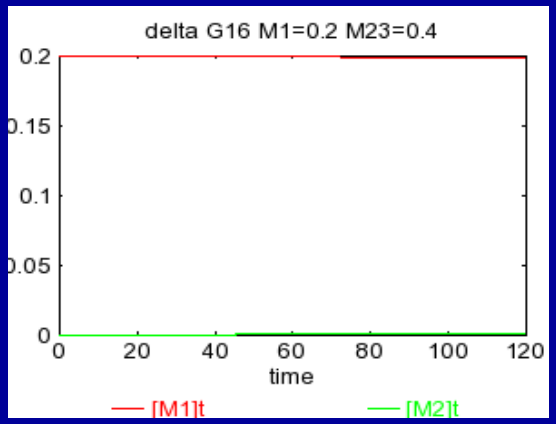

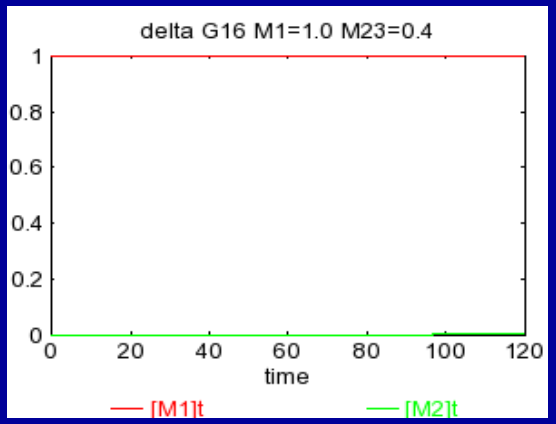

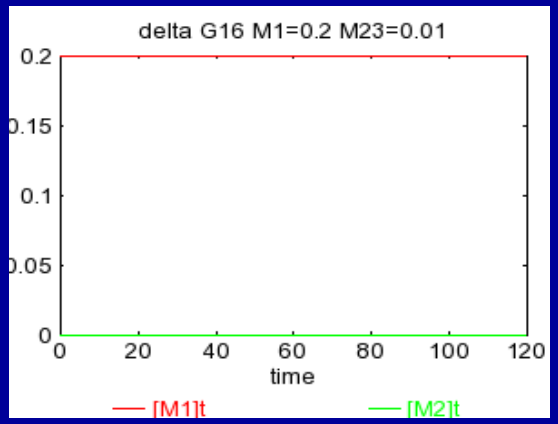

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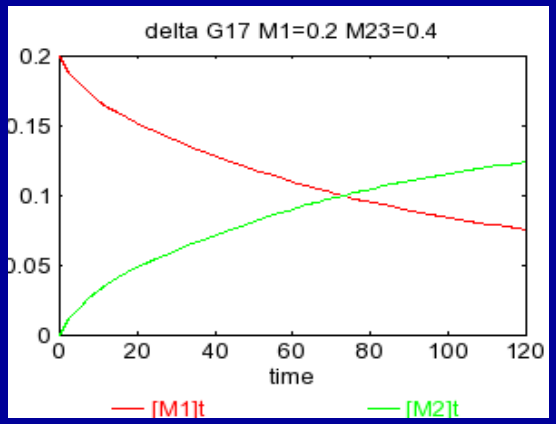

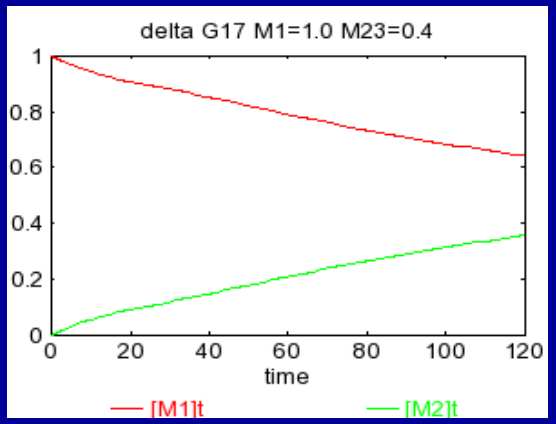

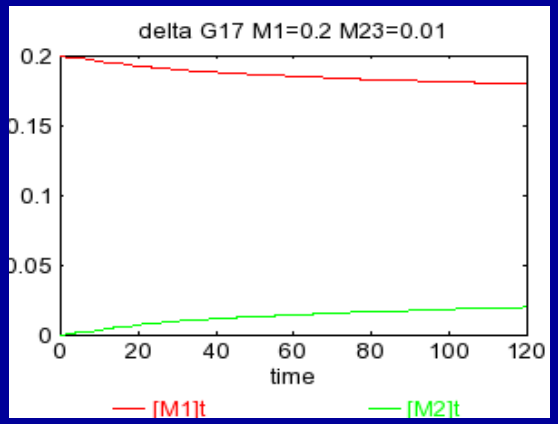

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STRAINS

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- ΔG16
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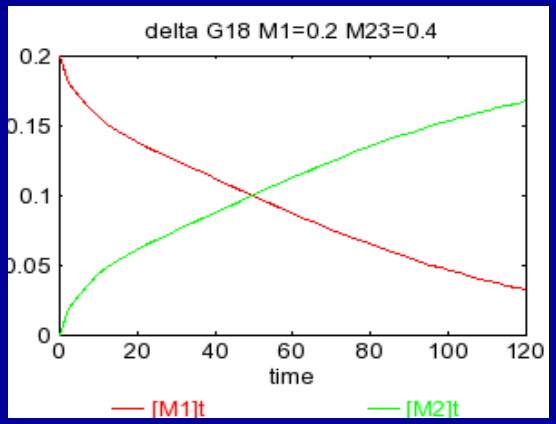

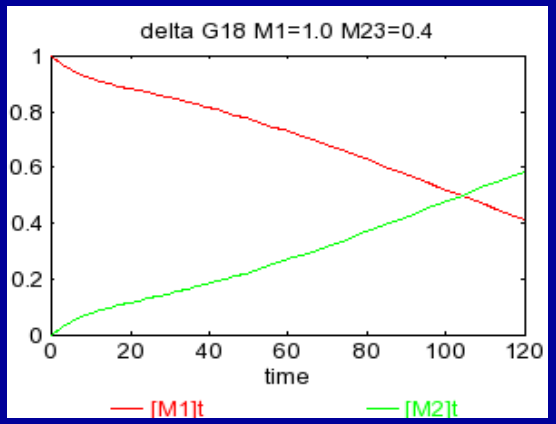

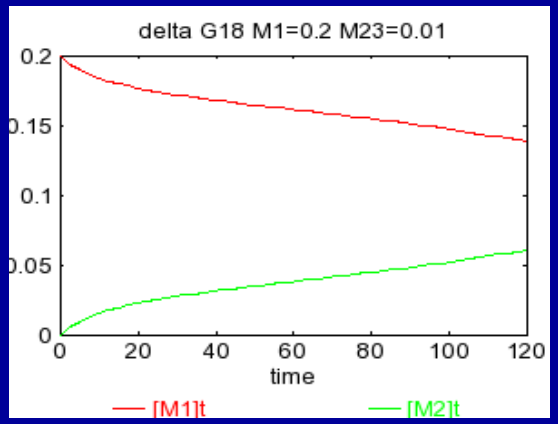

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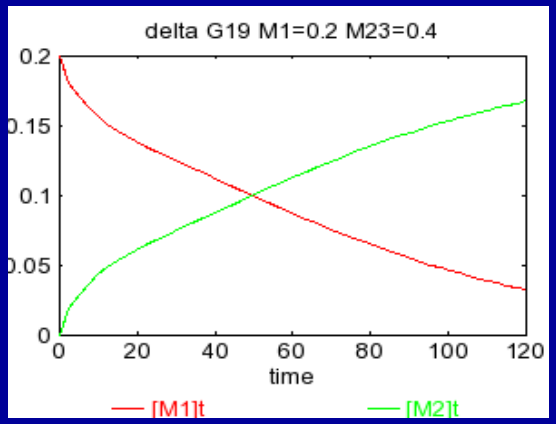

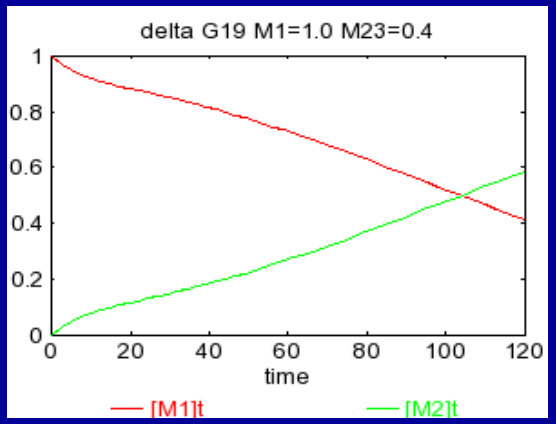

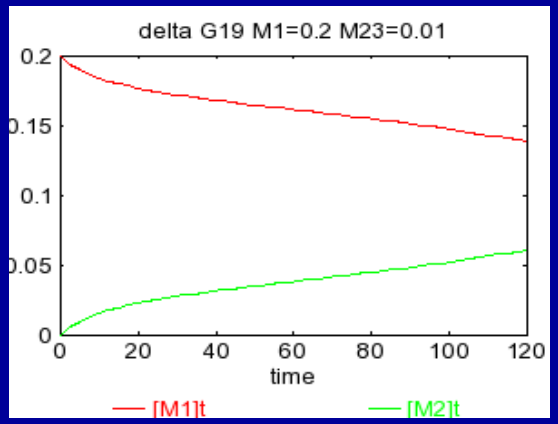

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Comparison of four reverse engineering methods

- RSA: de la Fuente, A., Brazhnik, P. & Mendes, P. (2002) Linking the genes: inferring quantitative gene networks from microarray data. *Trends in Genetics* **18**, 395-398
- NIR: Gardner TS, di Bernardo D, Lorenz D, Collins JJ (2003) Inferring genetic networks and identifying compound mode of action via expression profiling Science **301**, 102-105
- DBN: Yu J, Smith VA, Wang PP, Hartemink AJ, Jarvis ED. (2004) Advances to Bayesian network inference for generating causal networks from observational biological data. Bioinformatics **20**, 3594-603
- PC: de la Fuente, A., Bing, N., Hoeschele, I. & Mendes, P. (2004) Discovery of meaningful associations in genomic data using partial correlation coefficients. *Bioinformatics* **20**, 3565-3574

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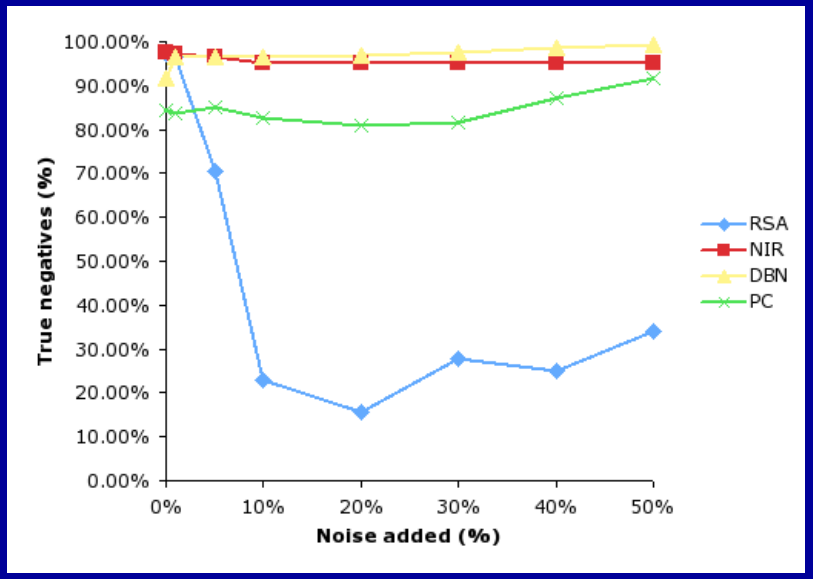
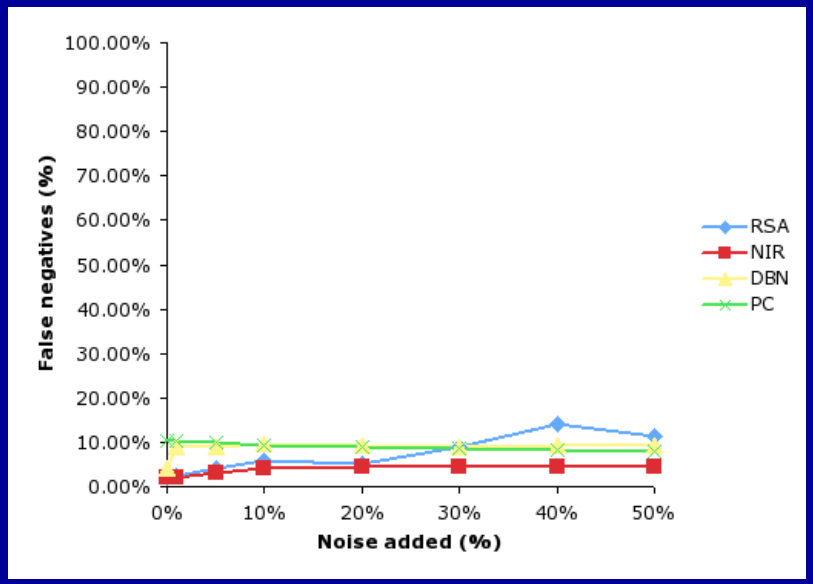
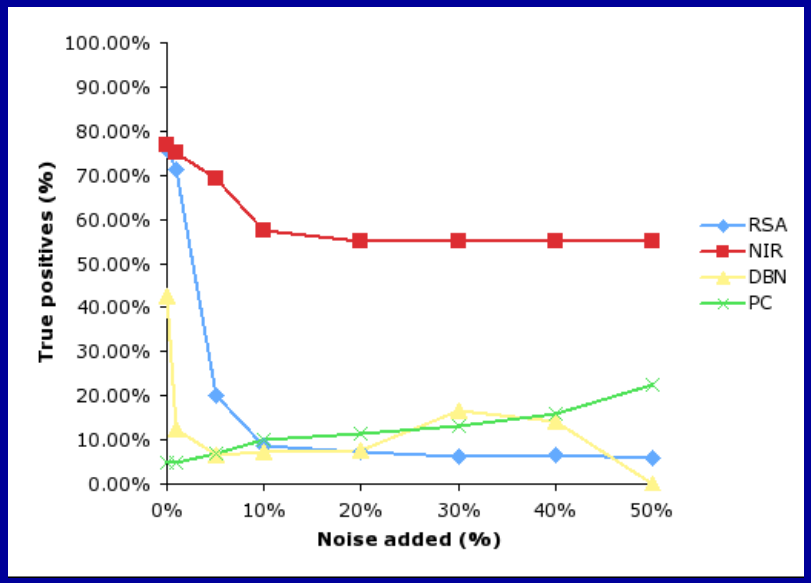
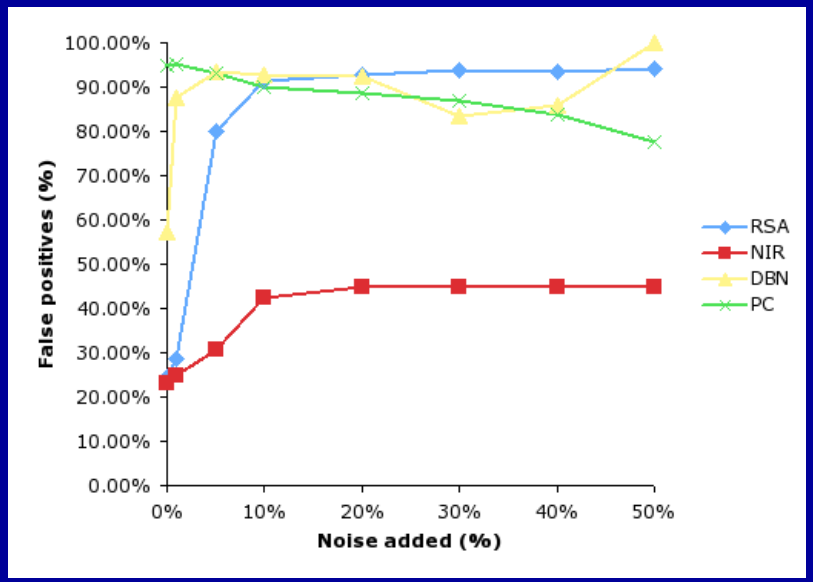
Increasing noise levels

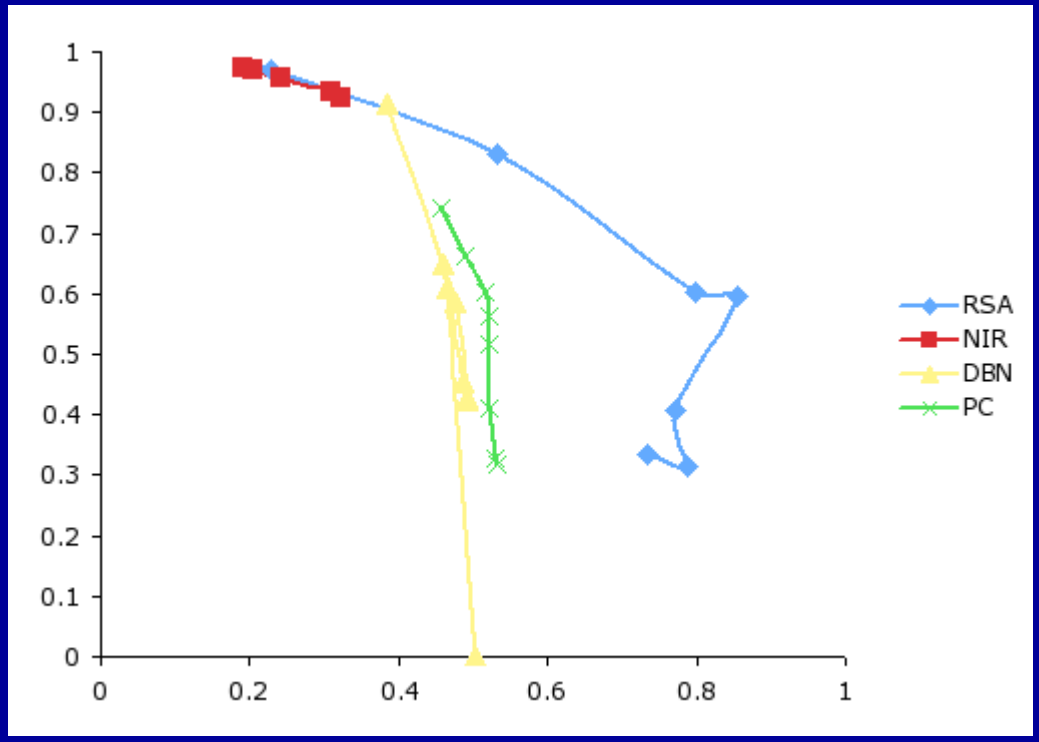








ROC curve for effect of noise



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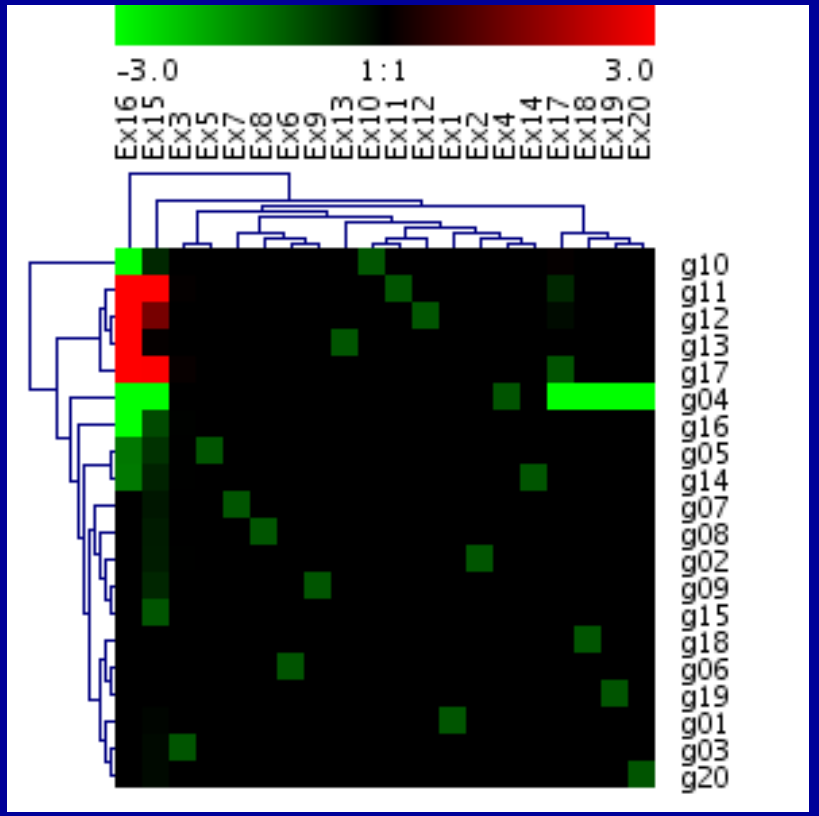
Heterozygous mutant experiments

- Each gene is mutated to have 50% translation rate
- Steady-state levels of all genes are measured and represented as ratio to *wt*
- Proteins and metabolites are not measured
- REALISM:
  - Response is a result of full biochemical network
  - Most mutations have little effect



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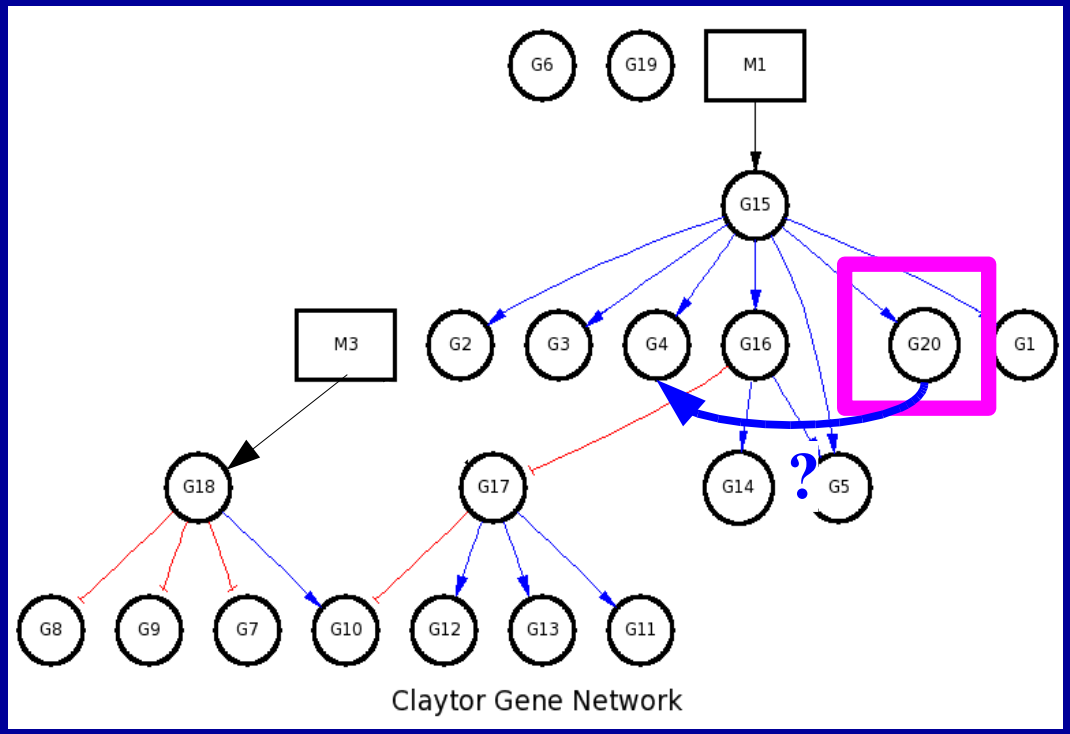
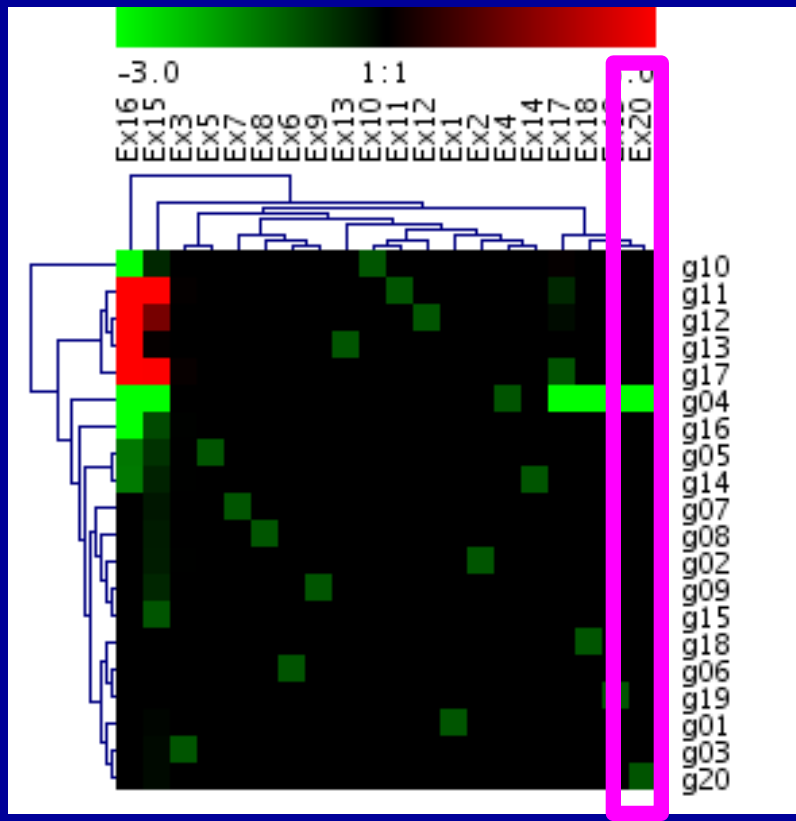
But do we really **know** the gene network accurately?





- Failure to identify all "connections" between genes
- No longer possible to calculate metrics accurately

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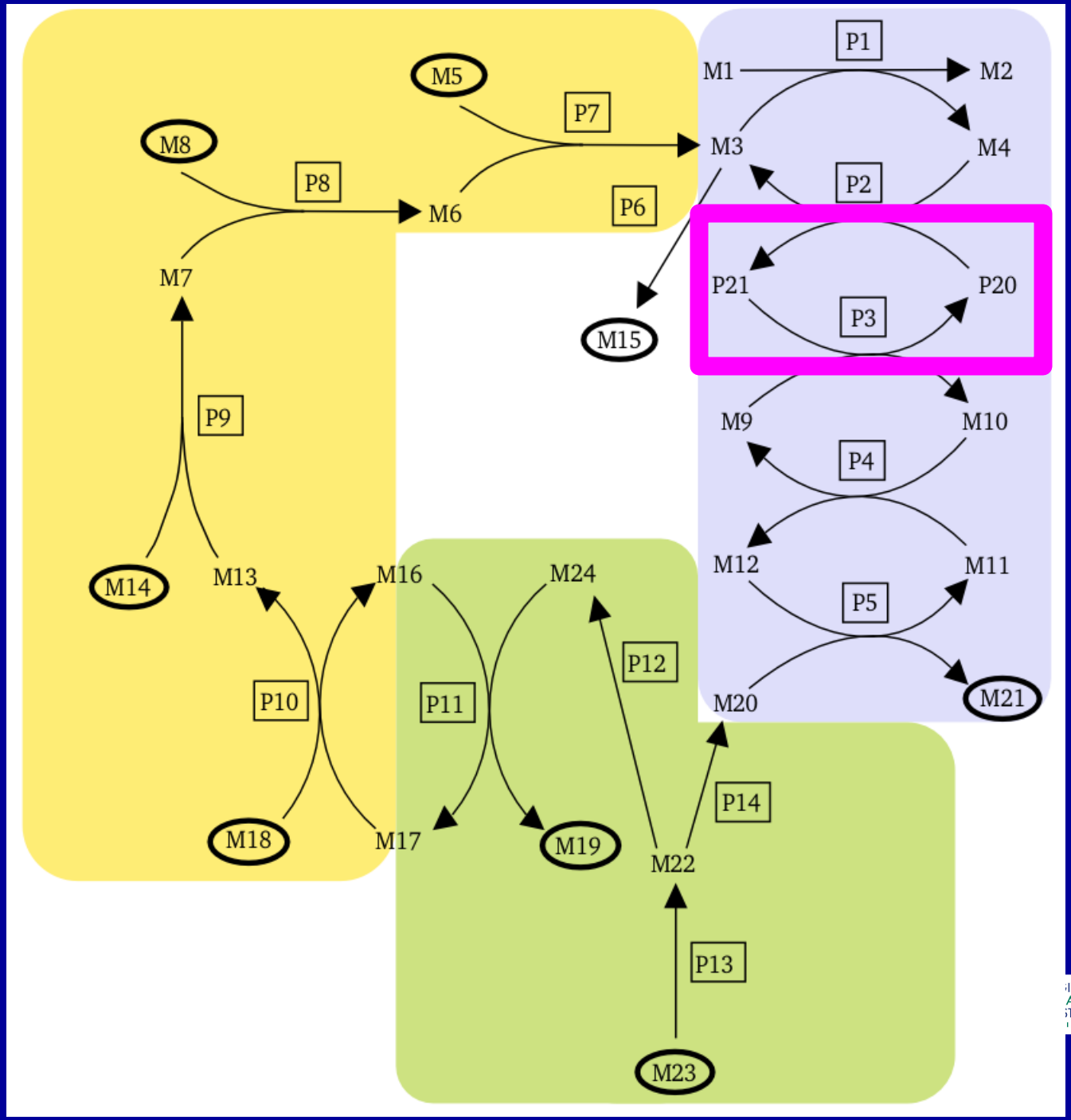
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P3
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M10
P4
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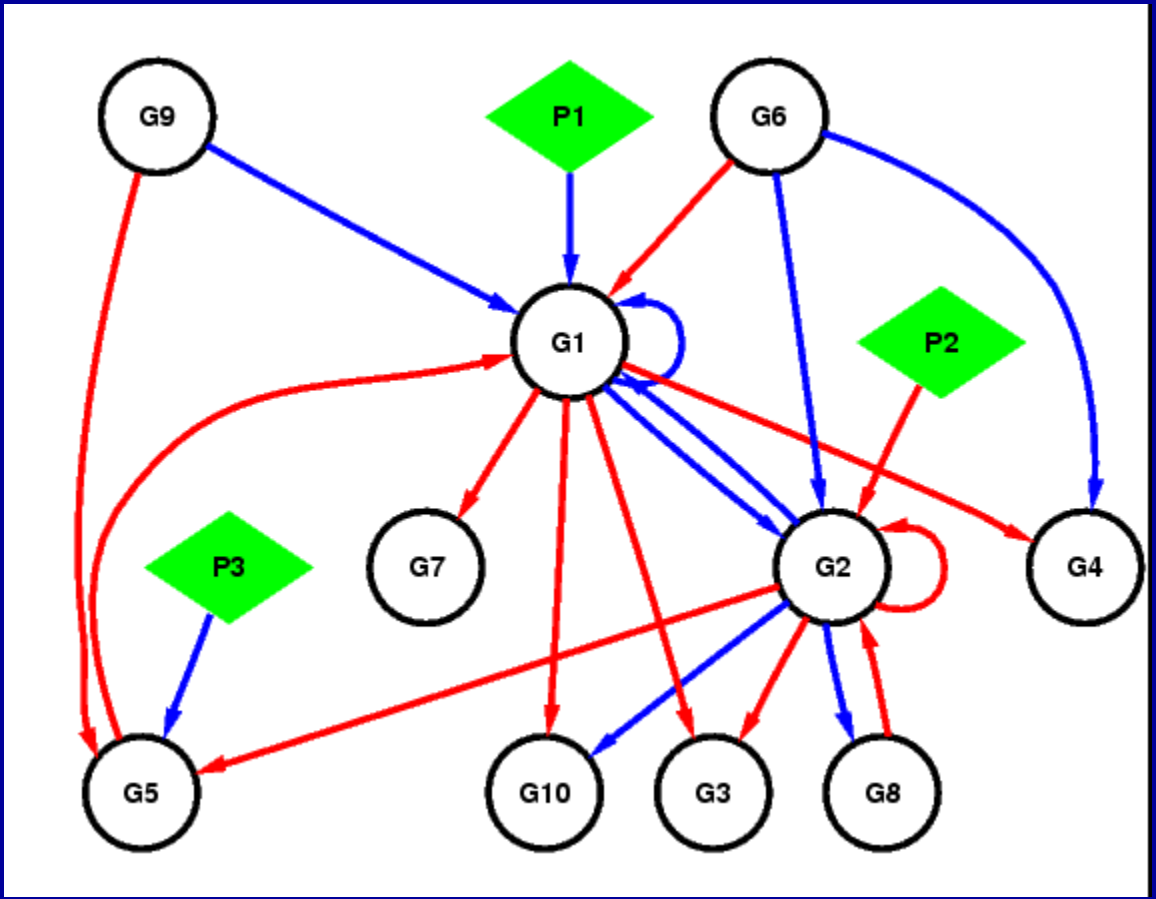
M20
M21

The Pandapas network

- Simple network with only 10 genes
- Same kinetic functions as in AGN
- Similar time scales for all genes
- Different range of values for each gene



Summary

- In silico networks provide challenging data for reverse engineering...
- They are well-suited to be gold standards
- The details of the simulated experiments may affect the possibility of having objective metrics

- In silico networks should be used for assessment of reverse engineering algorithms (but not exclusively!)

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Acknowledgements

Pedro Mendes
Diogo Camacho
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Aejaaz Kamal
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Bharat Mehrotra
Saroj Mohapatra
Revonda Pokrzwya

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