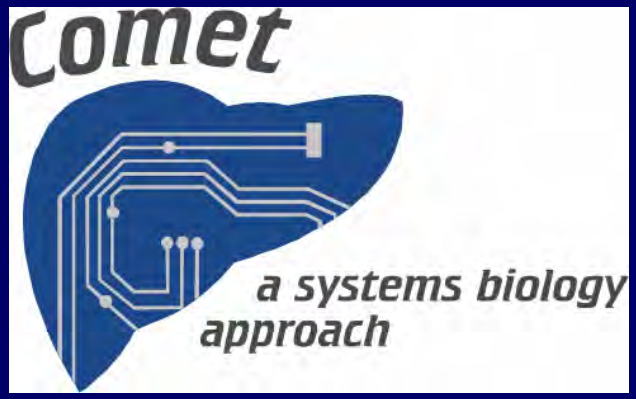
International Science Forum on Computational Toxicology
Research Triangle Park, NC
May 21-23, 2007

A quantitative understanding of dynamic cellular processes during detoxification in human hepatocytes

Matthias Reuss
Institute of Biochemical Engineering &
Centre Systems Biology
University of Stuttgart/Germany

Network Stuttgart

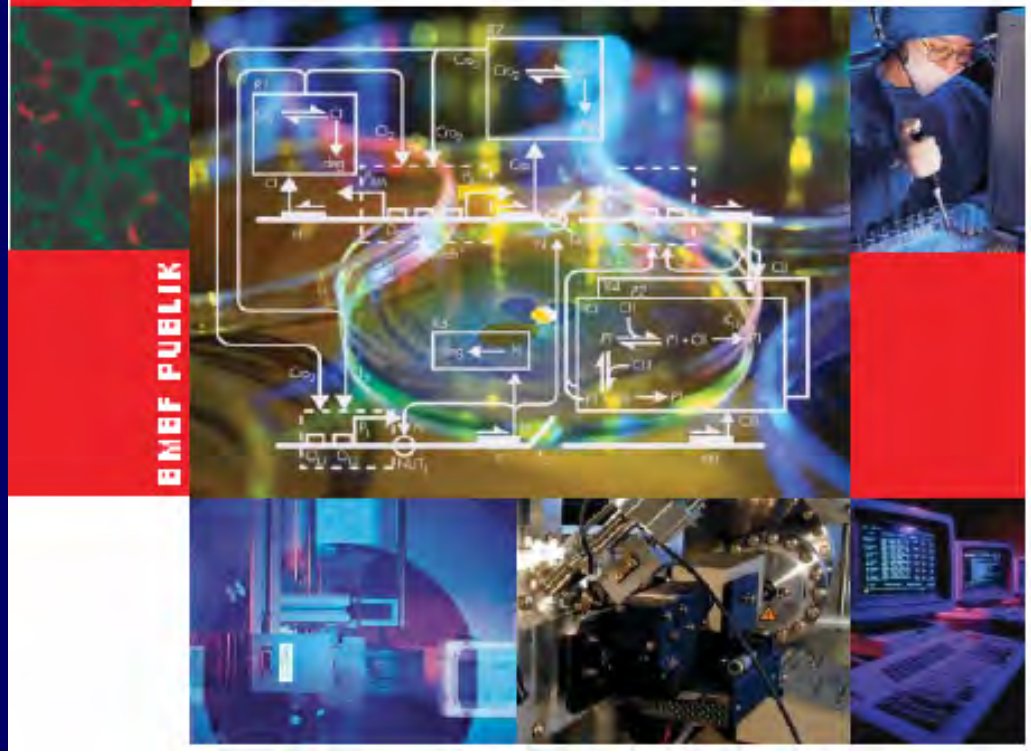
A Systems Biology Approach to Detoxification Processes in Hepatocytes



Federal Ministry
of Education
and Research

Systems of Life

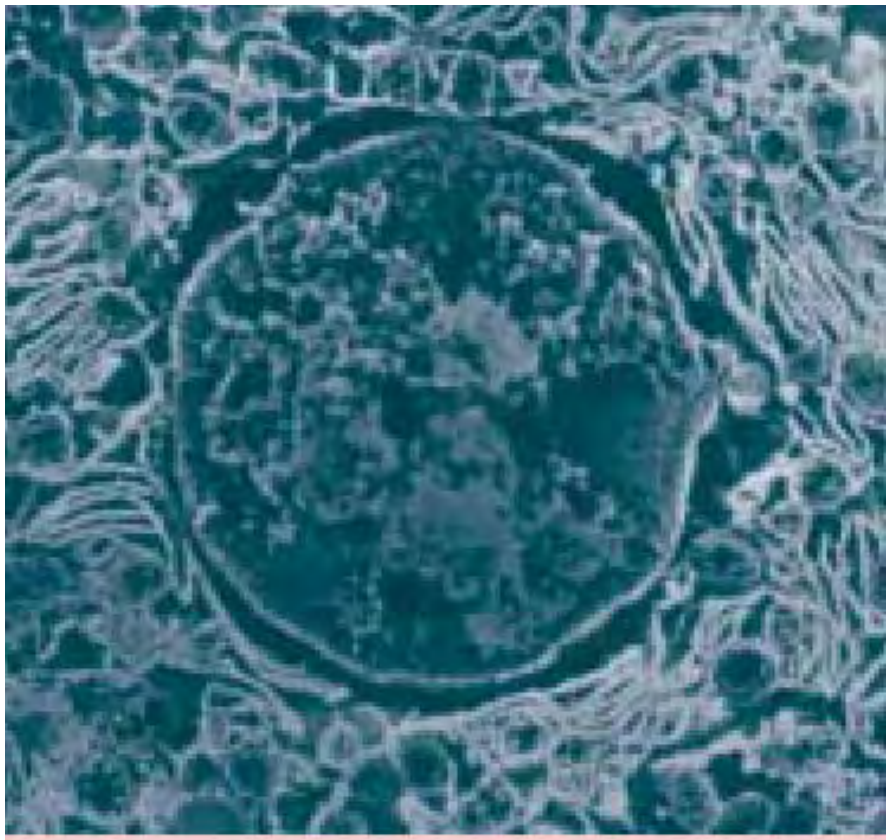
Systems Biology



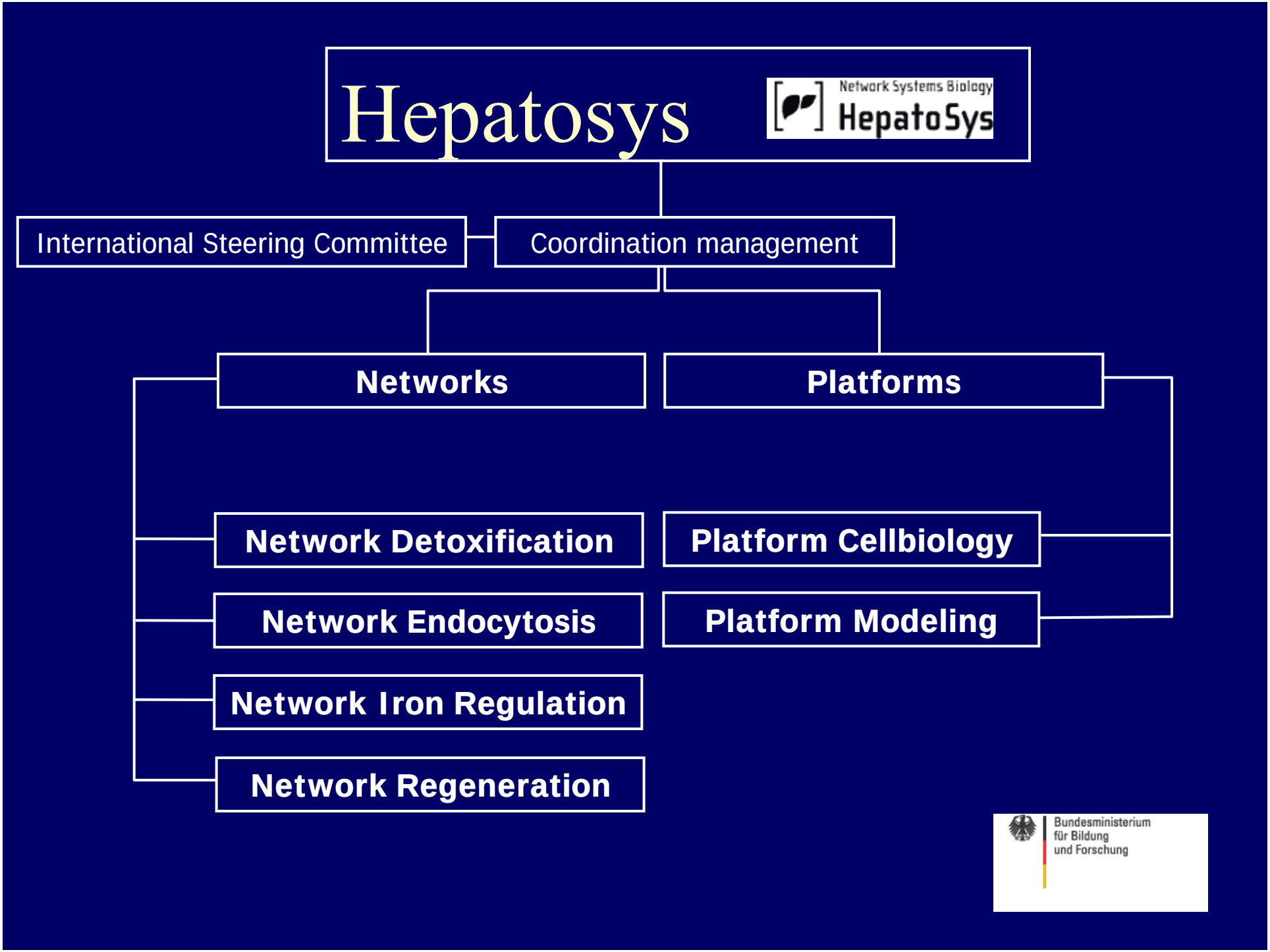
BMBF PUBLIK

Network Systems Biology

HepatoSys



Research under the Systems of Life – Systems Biology programme will focus on the hepatocyte system


Hepatosys
Network Systems Biology
HepatoSys
International Steering Committee
Coordination management
Networks
Platforms
Network Detoxification
Network Endocytosis
Network Iron Regulation
Network Regeneration
Platform Cellbiology
Platform Modeling
Bundesministerium
für Bildung
und Forschung

DETOXIFICATION

Holistic analysis, mathematical modelling and simulation of the detoxification system of the human hepatocyte

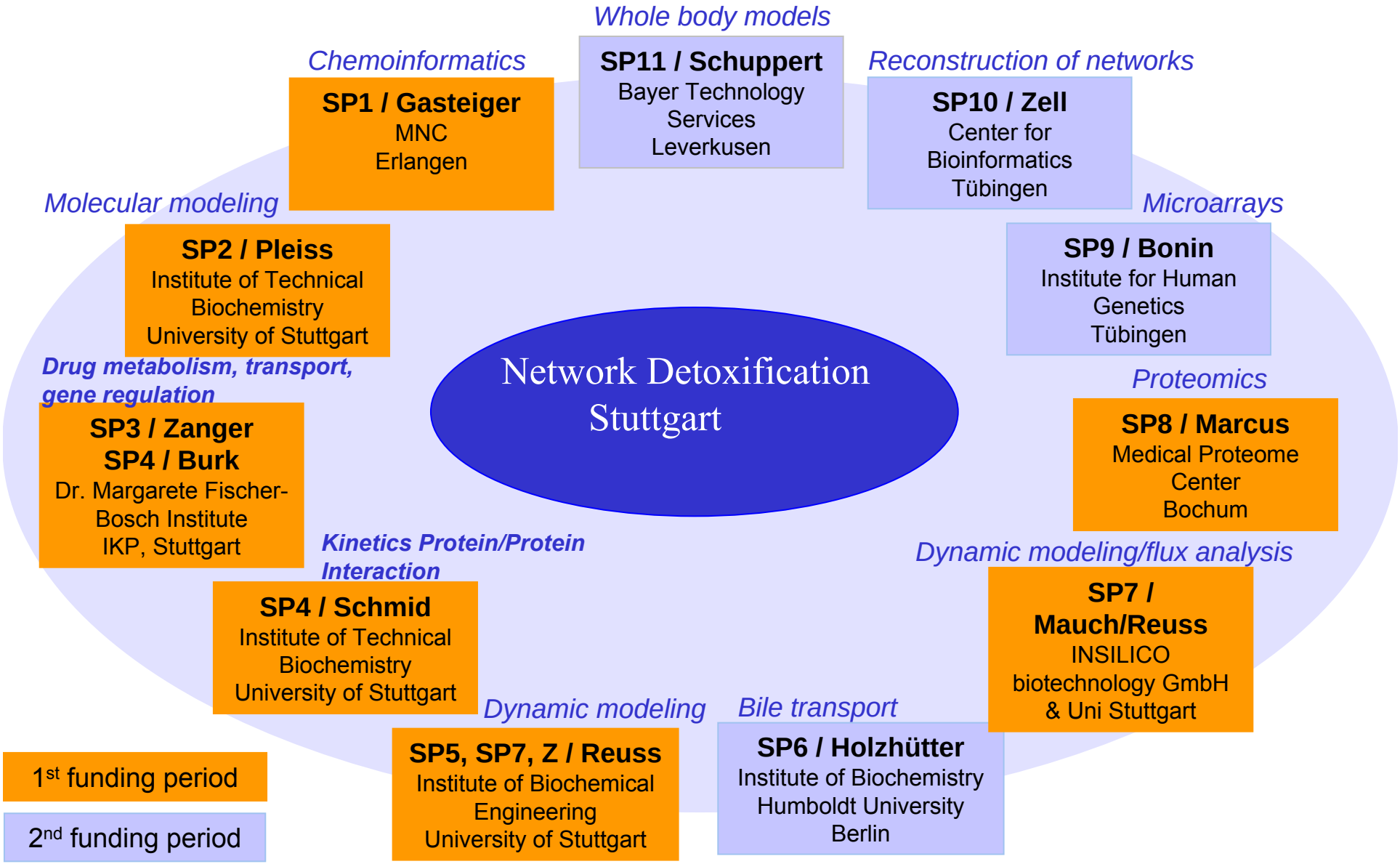
12 research projects: Universities Stuttgart (IBVT, ITB, ISA), Tübingen (Microarry Facility, Centre of Bioinformatics) Erlangen (Chemoinformatics), Bochum (Proteomics), Dr. Margarete – Bosch Institute Clinical Pharmacology, Stuttgart, Charitee/Humbold-University Berlin, INSILICO biotechnolology, Bayer Technology Service)

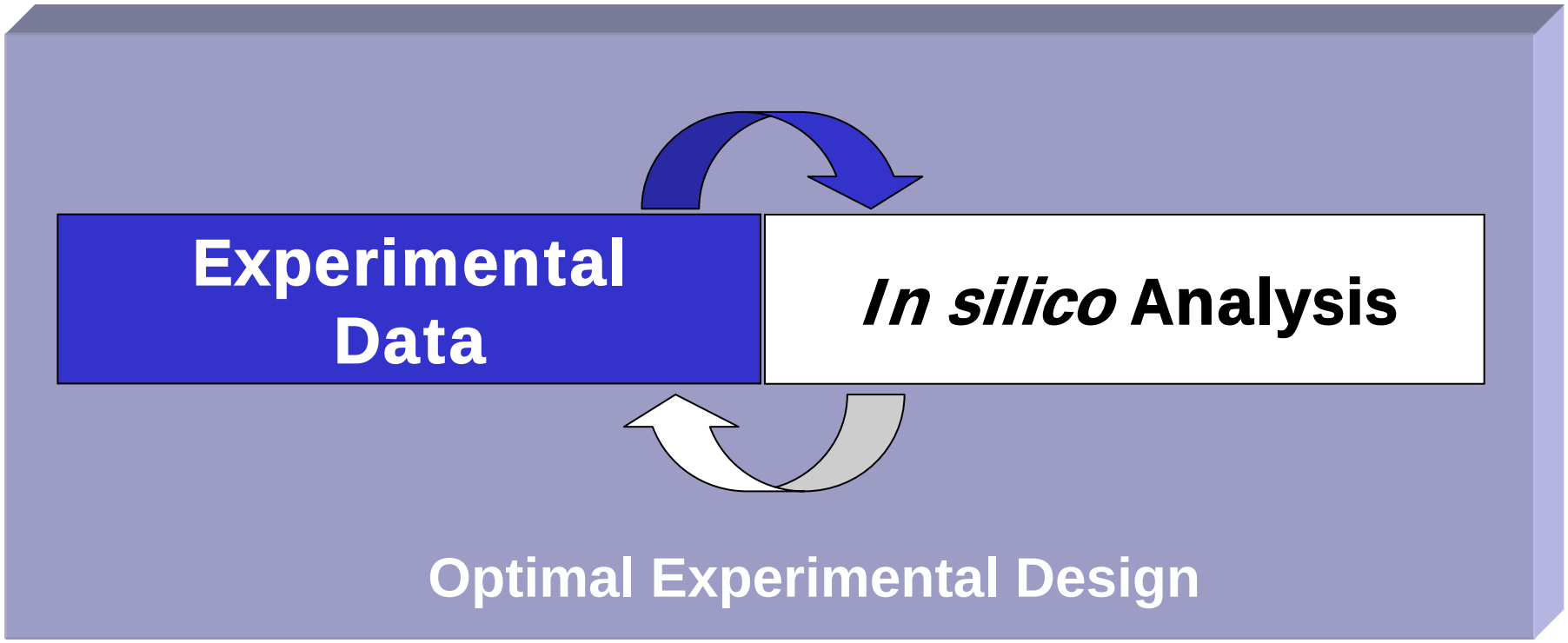
Chemoinformatic, Molecular Modeling,Dynamic Modeling of Detoxification,

Microarrays, Proteomics, Metabolomics

Modeling and simulation of signaling, regulatory and metabolic networks

Projectpartners Network Detoxification, Stuttgart 2007 - 2009




Experimental Data
In silico Analysis
Optimal Experimental Design

Dynamic modeling and simulation of the drug metabolizing P450 network

By placing the entire integrated network, rather then individual CYPs at the centre of dynamic analysis, a systems behaviour may emerge which differs from the reductionistic kinetics of individual enzymes.

ROBUSTNESS/FRAGILITY

STRUCTURAL AND DYNAMIC ROBUSTNESS

„Robustness enables the system to maintain its functionality against external and internal perturbations“ (*Kitano, 2004*)

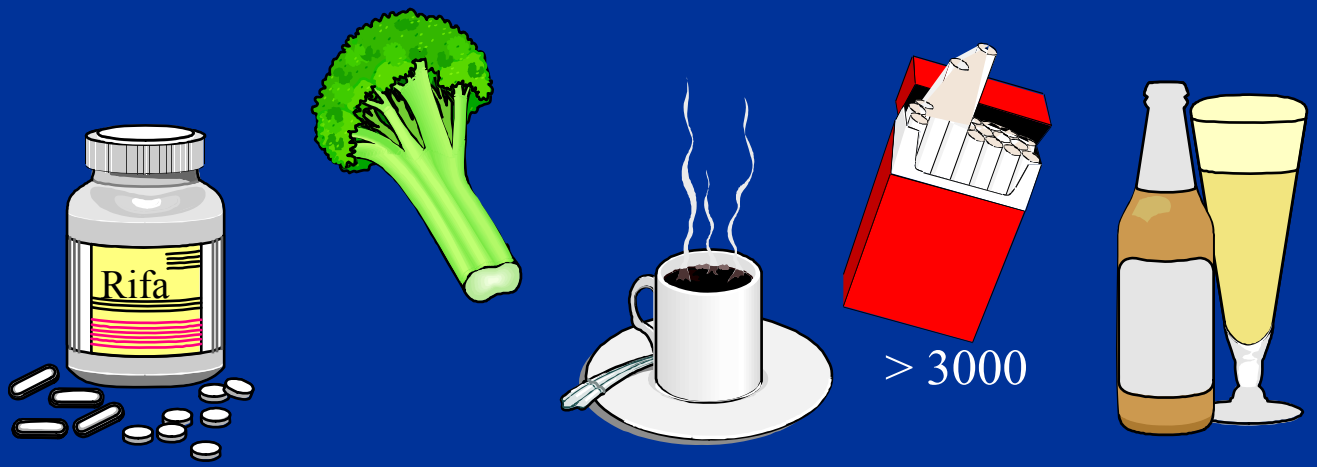
↓

Functionality:: Drug detoxification

External perturbation::multitude of substrates (varying concentrations and drugs)

Internal perturbation:: different enzyme expression levels; polymorphism, inter-individual variability → *phenotype plasticity*

Drug Metabolism: First Line of Defense Against Xenobiotics



Biosphere: ~ 3 million substances

Industry: ~ 3000 chemicals/year

Drugs: ~ 6000 (WHO)

Daily intake of xenobiotics: ~ 10.000 substances;

External perturbation

Internal perturbation

Pharmacogenetics

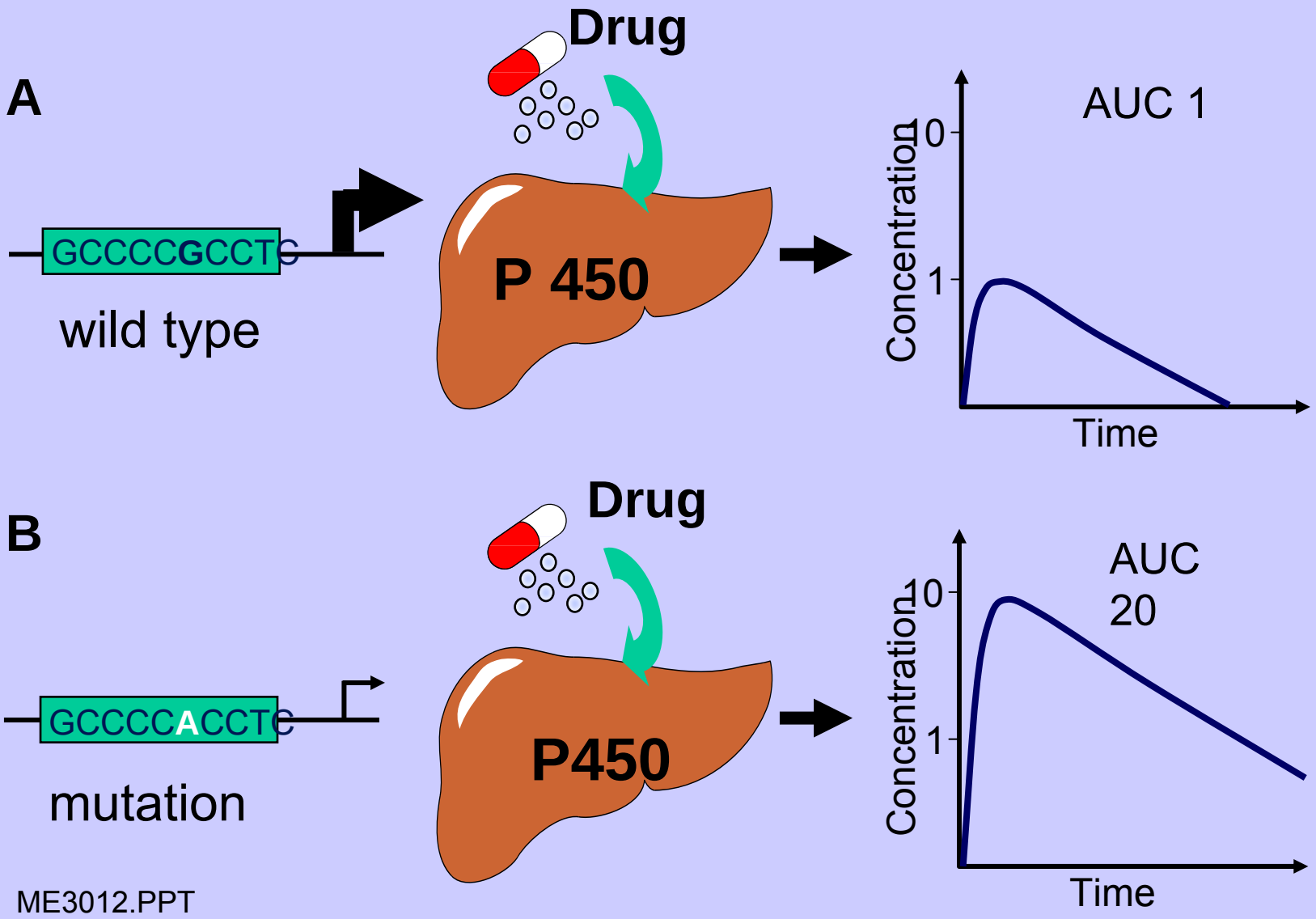
Individual variability in the efficacy and toxicity

of drugs due to polymorphisms of genes

involved in their disposition and action

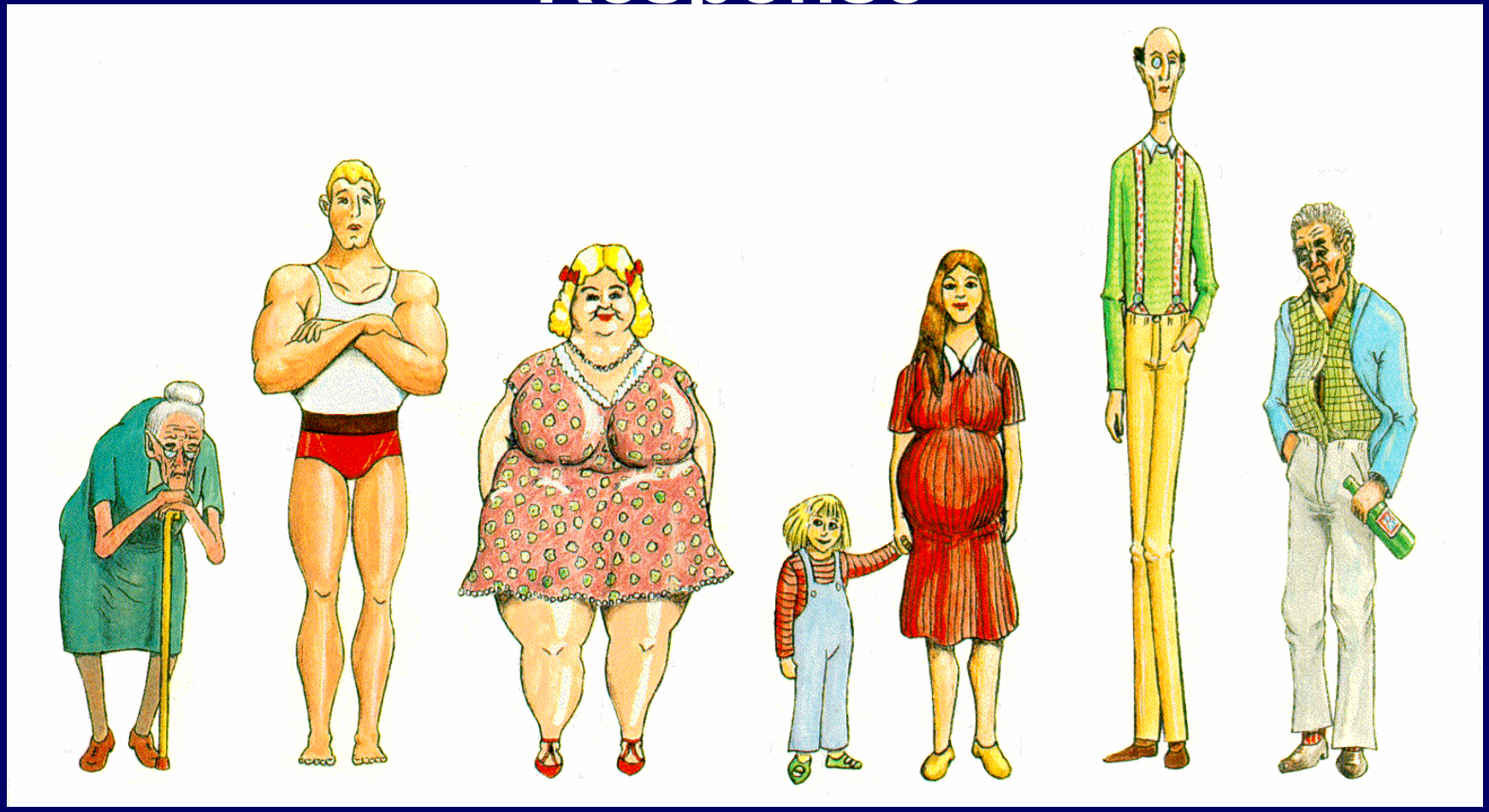
Mechanism of Genetic Variability in Drug Response

Same dose but different plasma concentrations



ME3012.PPT

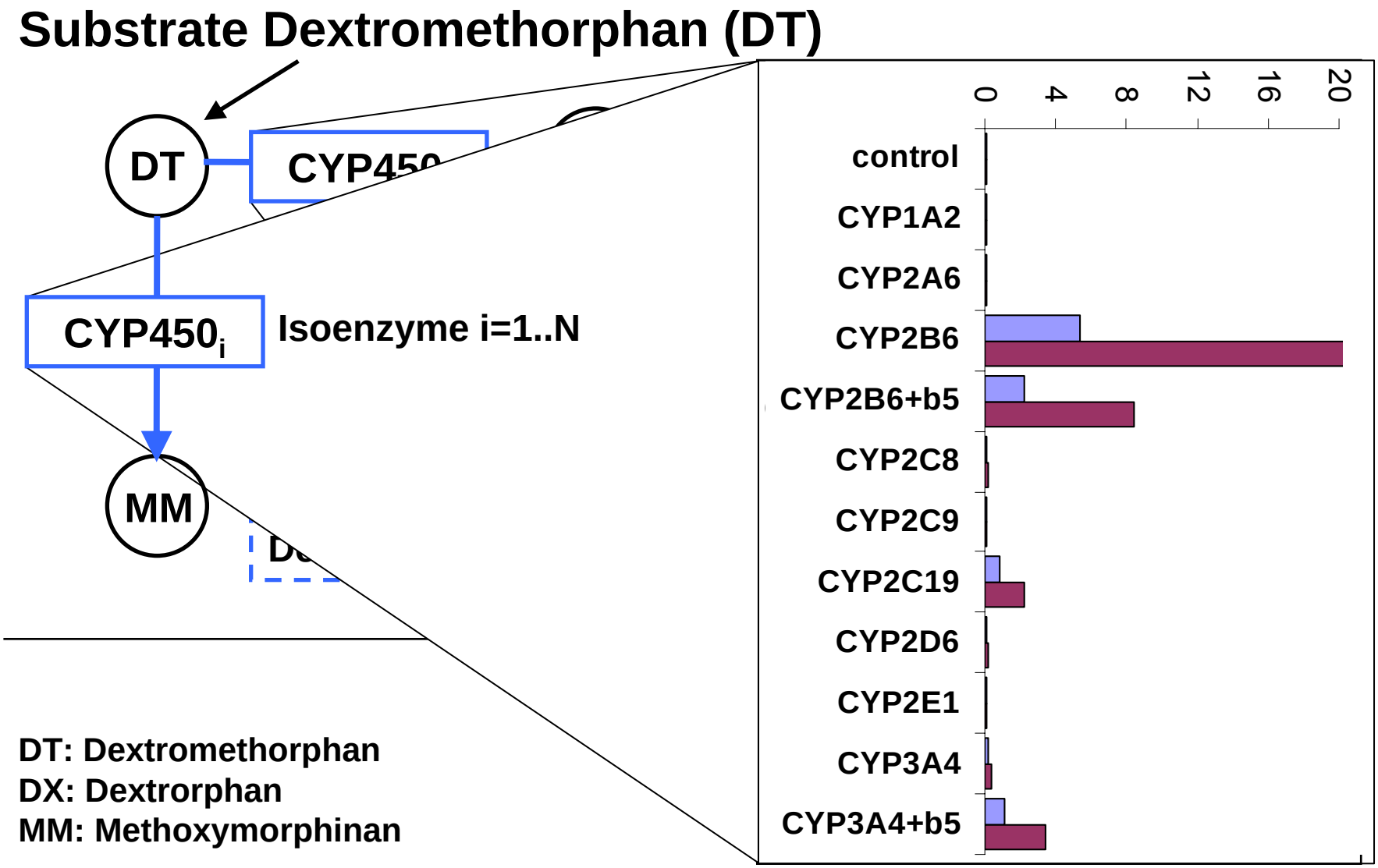
Nongenetic Factors and Drug Response



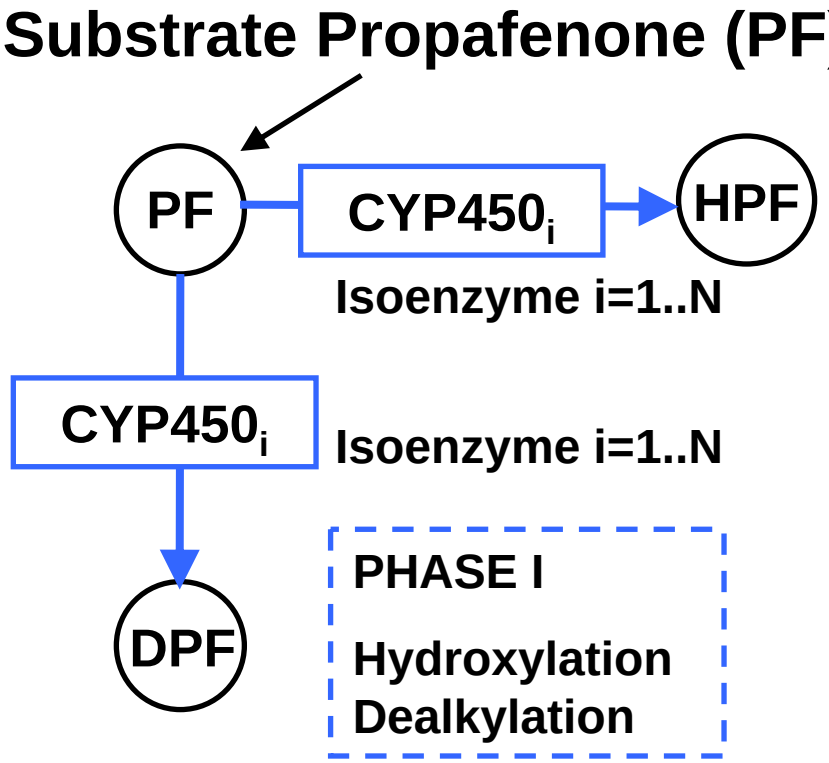
Age, body weight, sex, disease, diet, alcohol, smoking, drugs, hepatic and renal function

Karolisnska, Stockholm

Modeling: Pathway Identification



Modeling: Pathway Identification



PF: Propafenone
DPF: Desalkyl-Propafenone
HPF: 5-OH-Propafenone

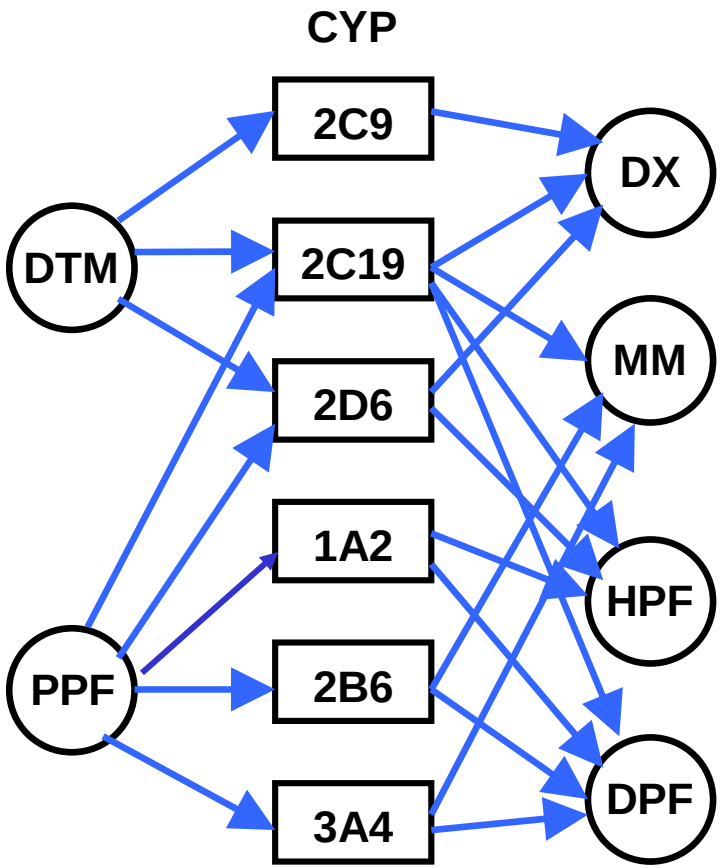
CYP450: Cytochrome P450 monooxygenase

Model Set-up

Multireactant isoenzyme system

Dextromethorphan

Propafenone



Model: Set-up

Multireactant isoenzyme system

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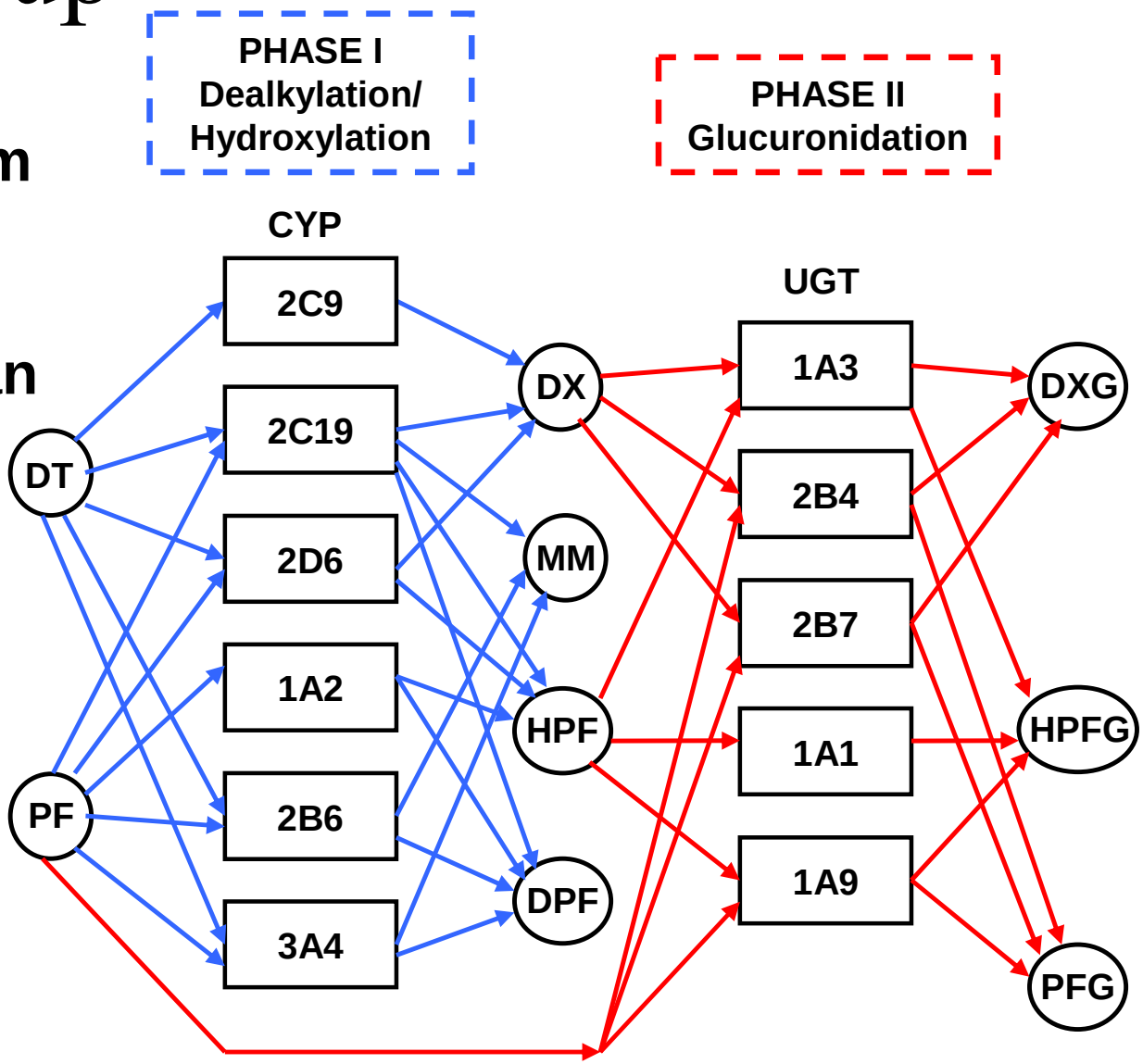
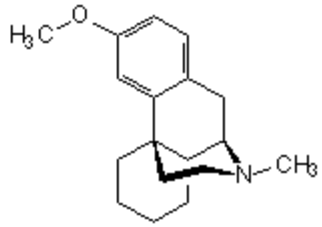
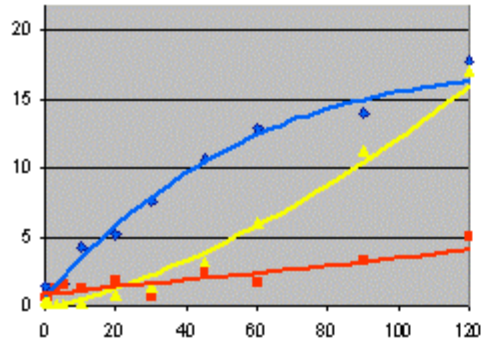


Quantitative LC-MS/MS Analysis of Drugs and Metabolites

Zanger et al., Clinical Pharmacology, Rober Bosch, Stuttgart

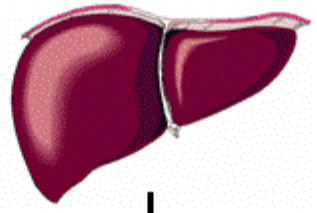
- synthesis of reference compounds and of **stable isotope-labeled analogs**
- method development and validation for quantification from various biological sources
- simultaneous quantitative analysis of drugs and metabolites (phase I / II) by LC-MS/MS
- structure determination of unknown metabolites ($LC\text{-}MS^n$)

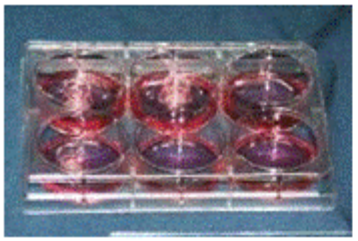


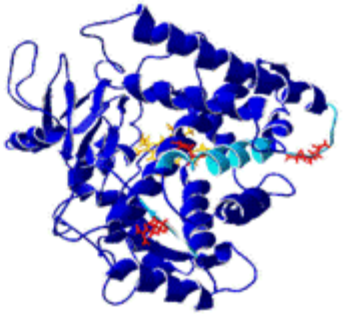


experimental approaches

complementary approach to human liver





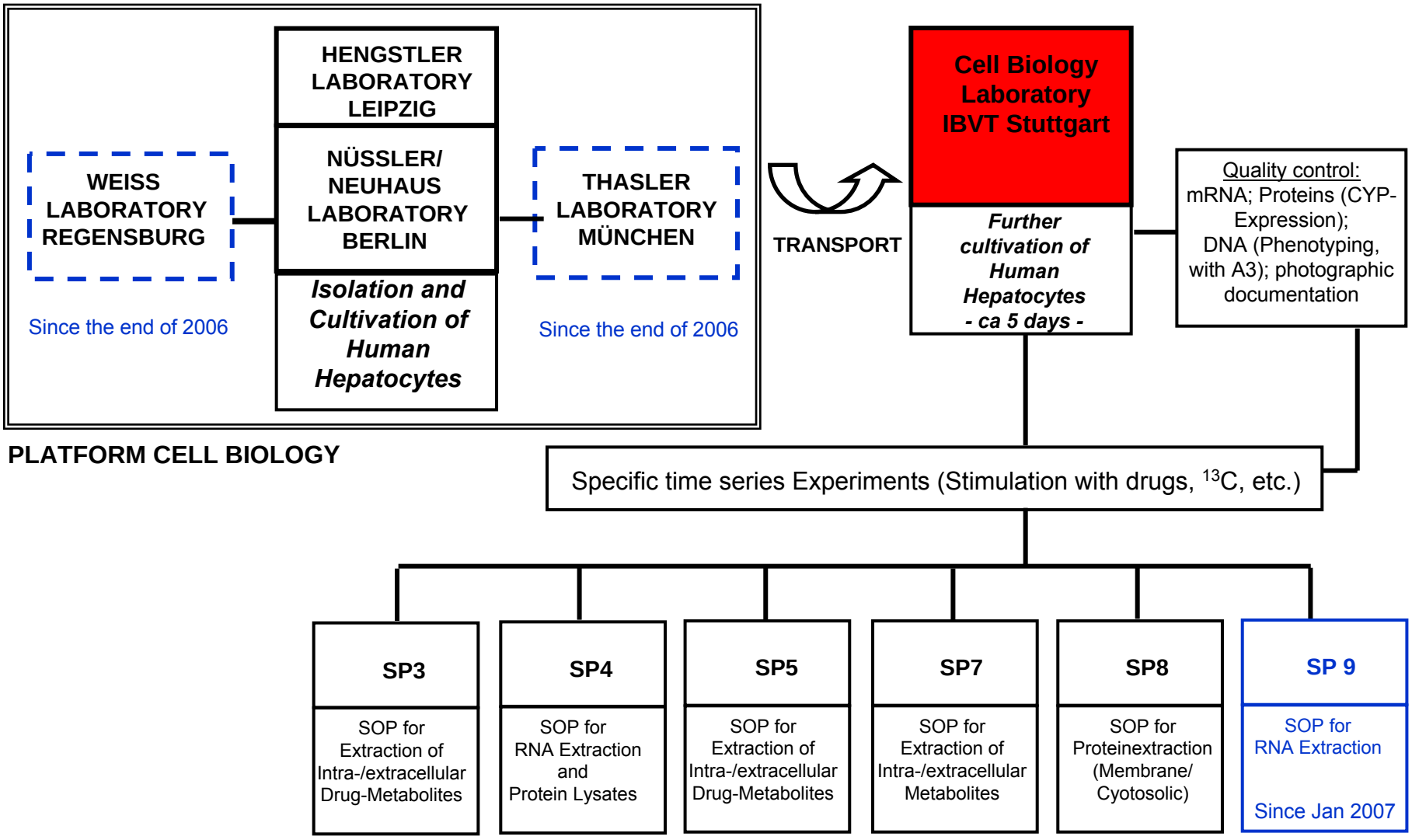


primary hepatocytes

liver tissue bank (N=300)

recombinant systems

Experimental work with primary human hepatocytes - logistics



Mathematical Model

Dynamic Balance Equations

$$\frac{dc_j}{dt} = \sum_i \upsilon_{i,j} r_{i,j}(t) = f(c(t), p) \qquad i = 1..N \quad \textit{Enzymes, } j = \textit{Metabolite}$$

Reaction rates: irreversible MM with multiple competitive inhibitions through alternative substrates

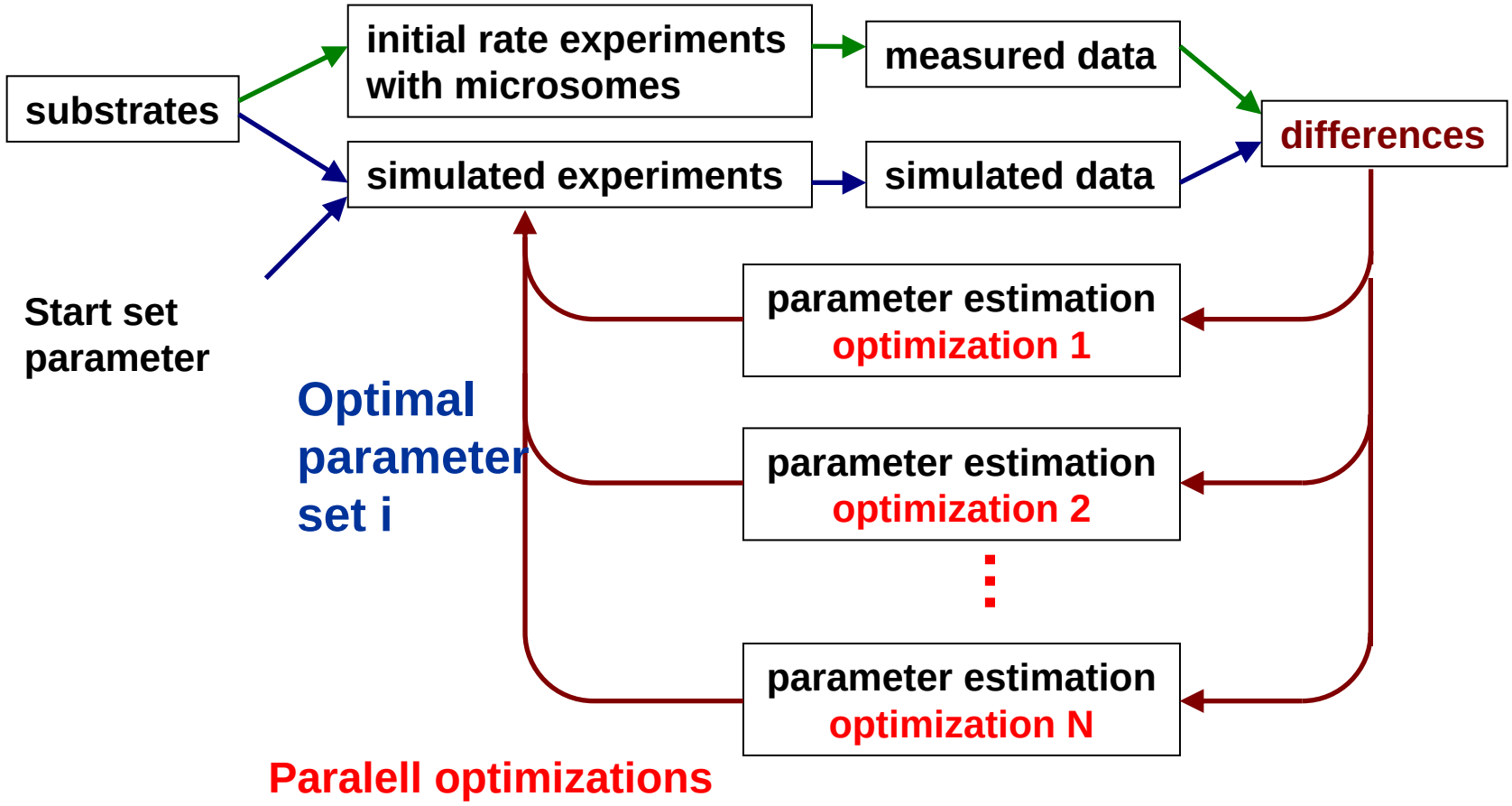
$$r^{Enz}_{S1\to P1} = \frac{r^{Enz}_{max,S1\to P1}\,\dfrac{c_{S1}}{K^{Enz}_{M,S1\to P1}}}{1 + \sum_j \dfrac{c_{S1}}{K^{Enz}_{M,S1\to Pj}} + \sum_k \dfrac{c_{S2}}{K^{Enz}_{M,S2\to Pk}}}$$

$$r^{Enz}_{max,S1\to P1} = k^{Enz}_{S1\to P1} \cdot \boxed{c^{ENZ}}$$

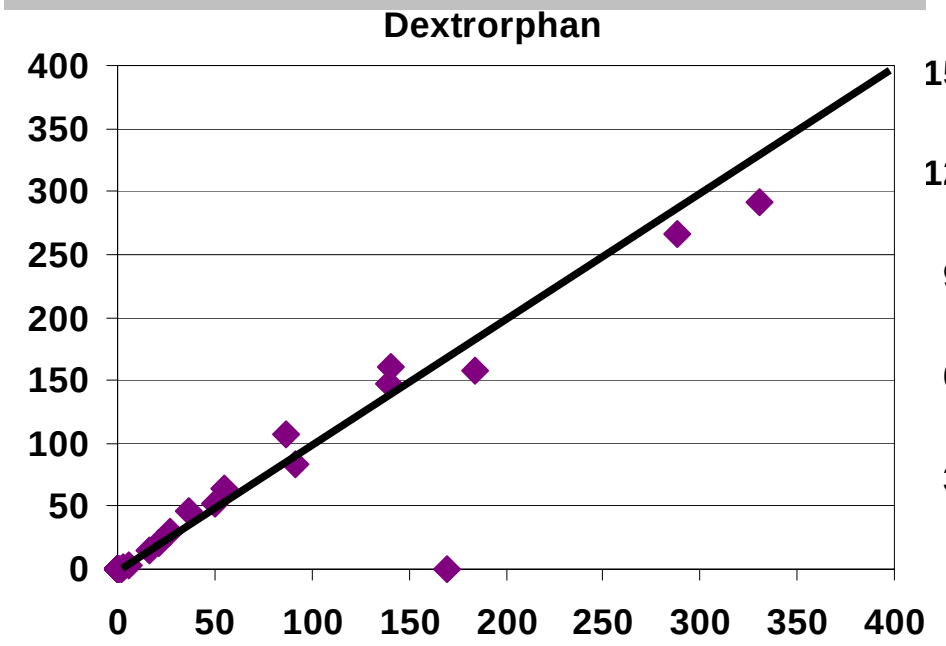
Quantitative Proteomics (labeled peptides, MPC Bochum)

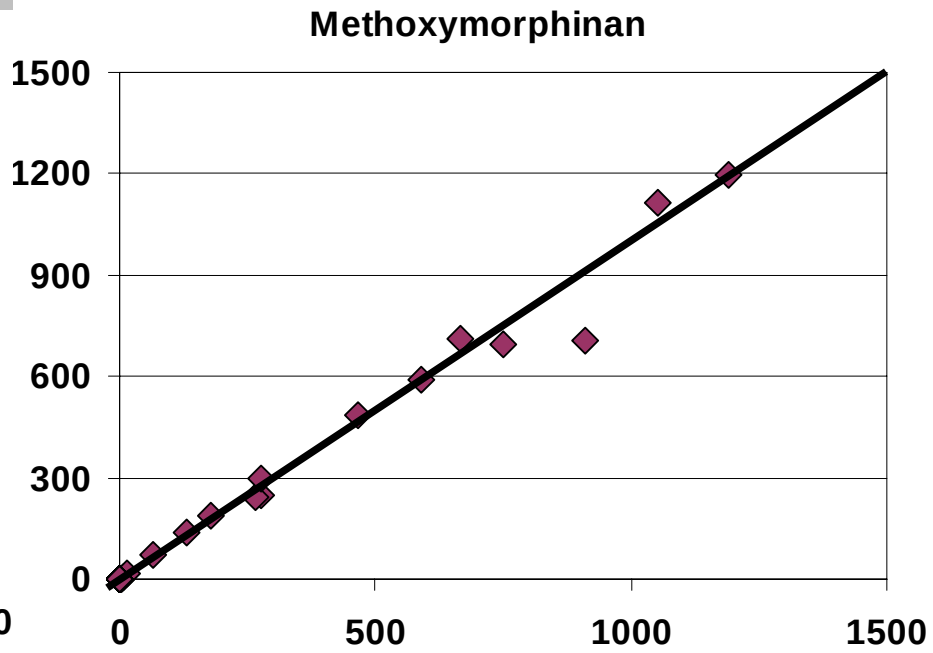
Parameter Identification

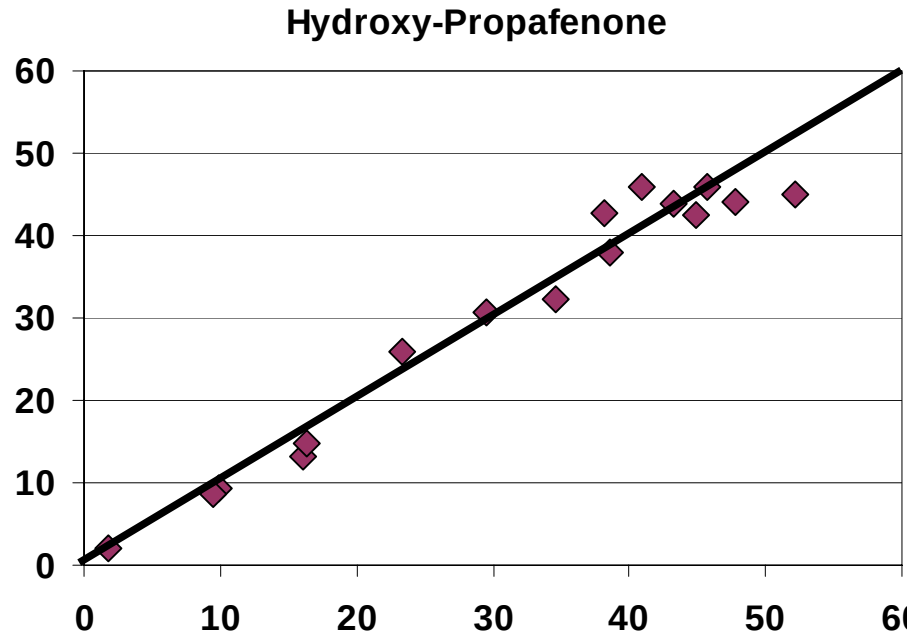
Optimization workflow

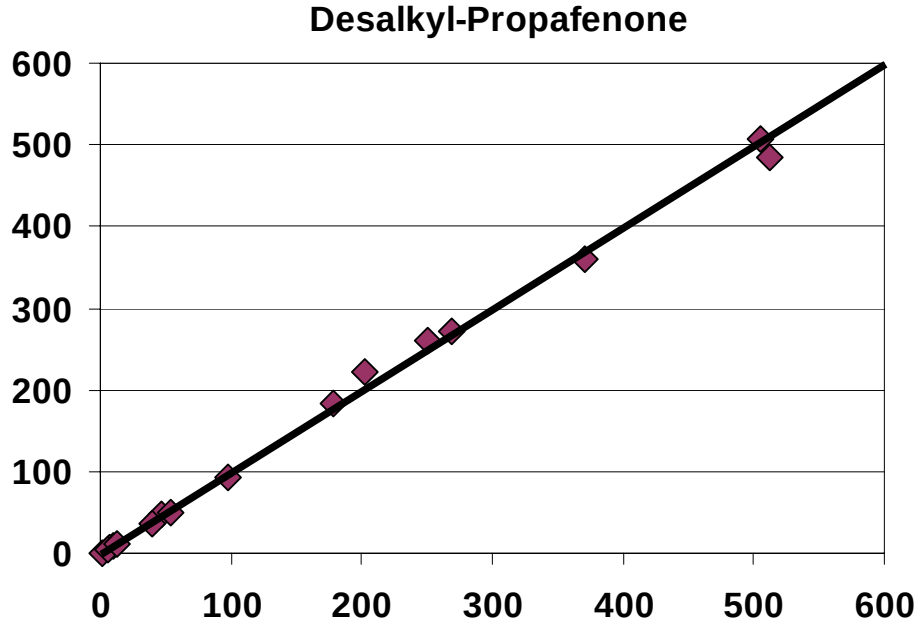


Parameter Identification





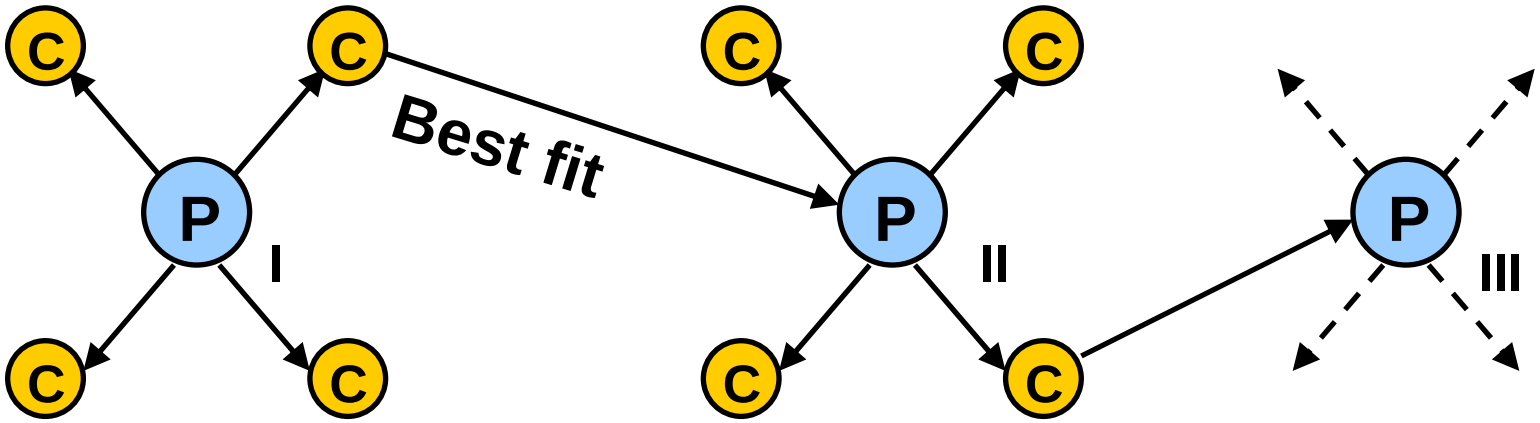




Parameter Identification

Evolutionary Algorithm (JavaEva[1]):

Generation of parameter set,
(c)hildren from (p)arents, via random process

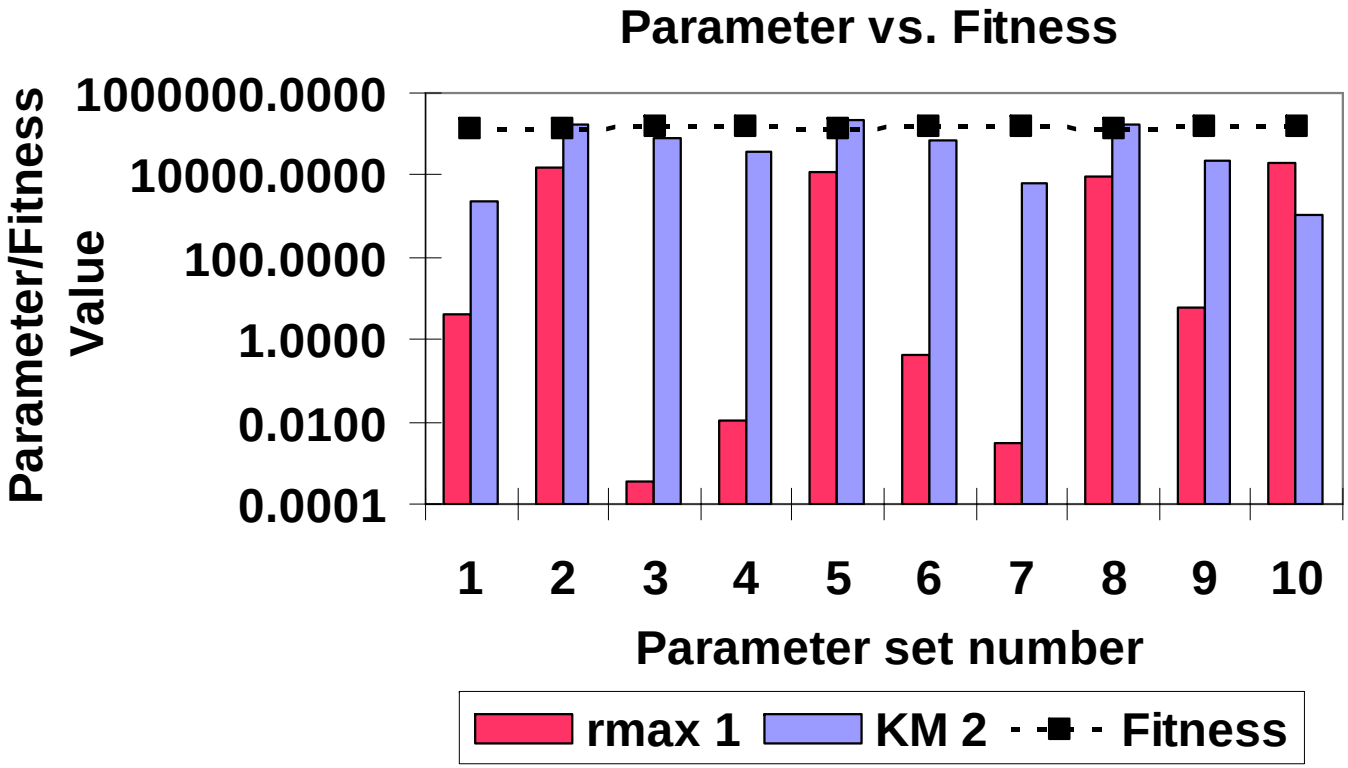


Parallel optimization with different random numbers (variation of parameters)

[1] **Prof.A.Zell, Center of Bioinfomatics, University of Tübingen**

Parameter Quality

Isoenzyme Model



Total average of relative deviation of parameters	**82 %**
Average Fitness	**143705.3**

Parameter Quality

Isoenzyme Model

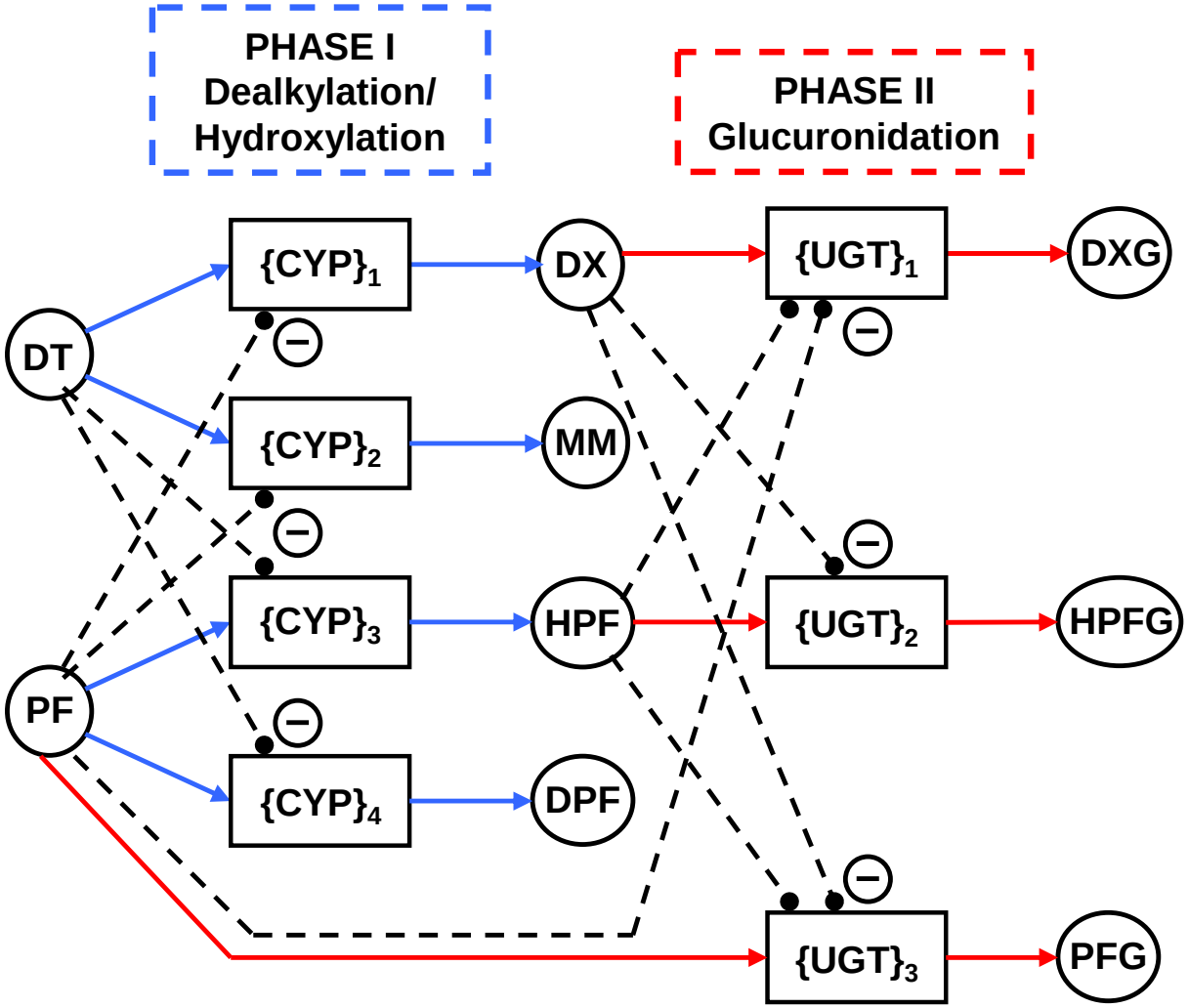
Parameter variation is very high

=> Indication of Robustness of the system

- **Model reduction feasible?**

Model Reduction

Superposition of isoenzymes per reaction, respectively



Number of parameters:

40 -> 22

Number of reactions:

22 -> 7

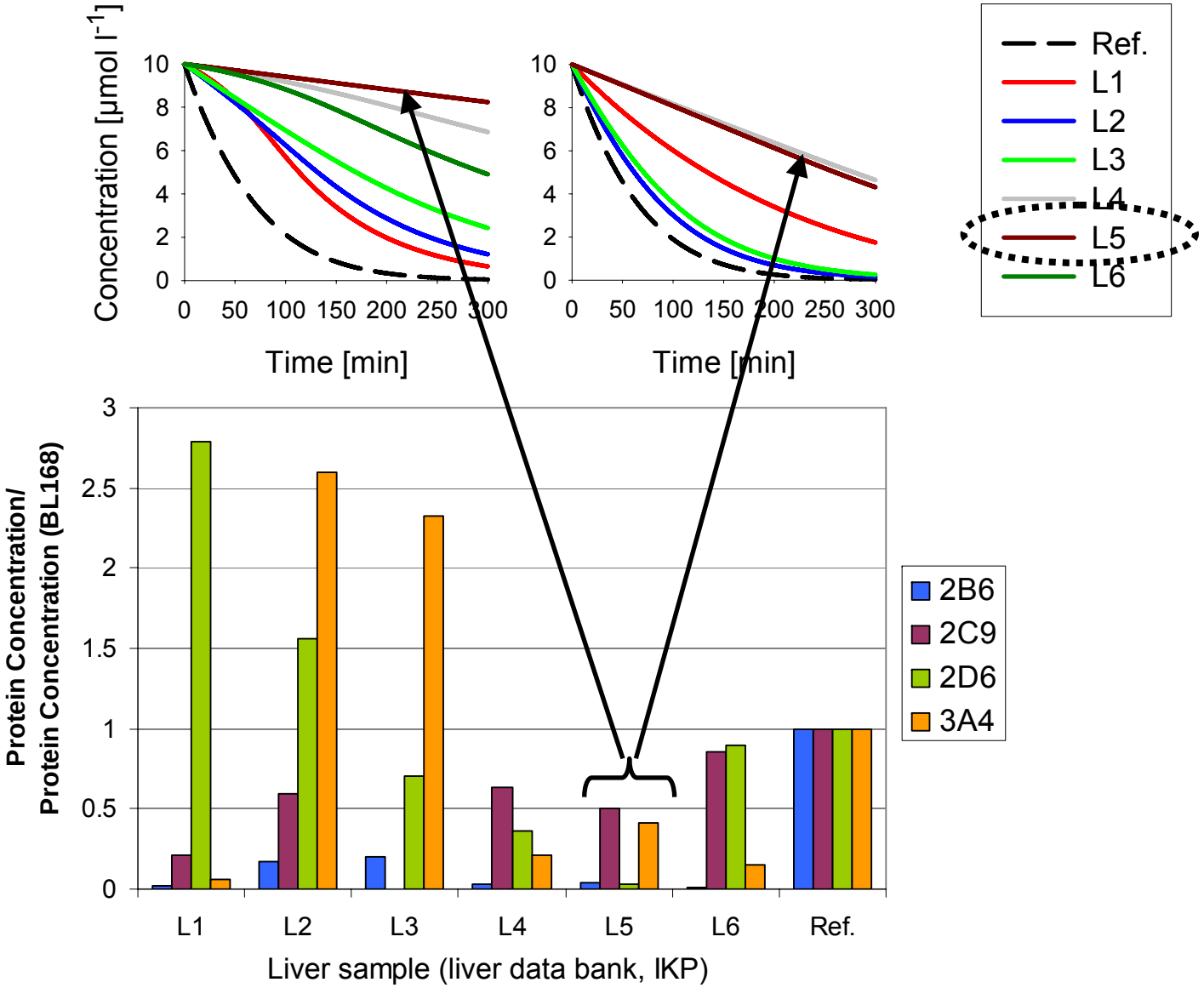
Total average of relative deviation of parameters:

82 % -> 18%

Fitness

143705.3 -> 175253.4

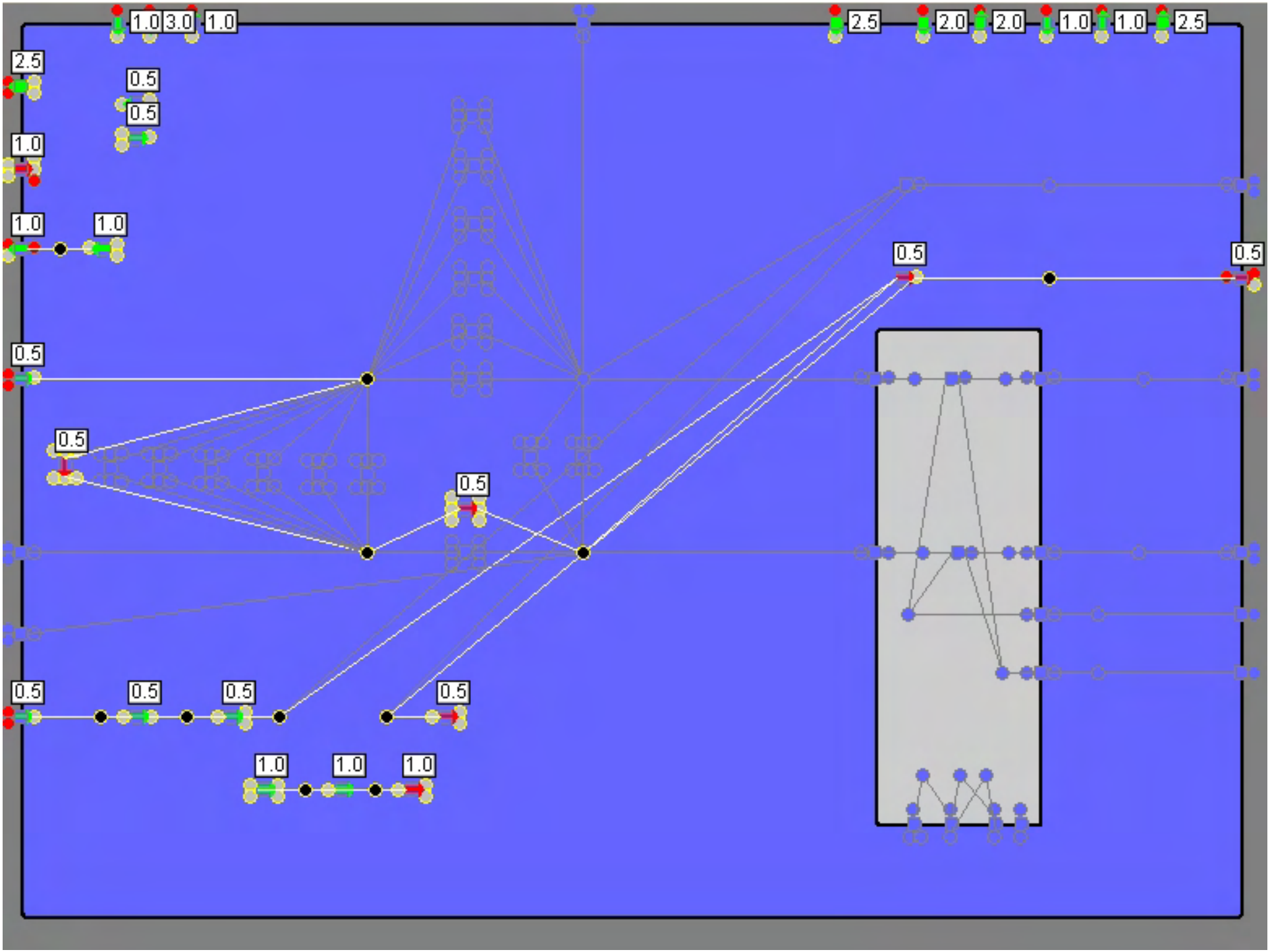
Dynamic Analysis: Drug Detoxification



STRUCTURAL ROBUSTNESS

ELEMENTARY FLUX MODES

- **Fulfil steady state condition**
- **Fulfil reversibilty properties**
- **Cannot be decomposed into smaller modes (i.e. modes that involve less enzymes)**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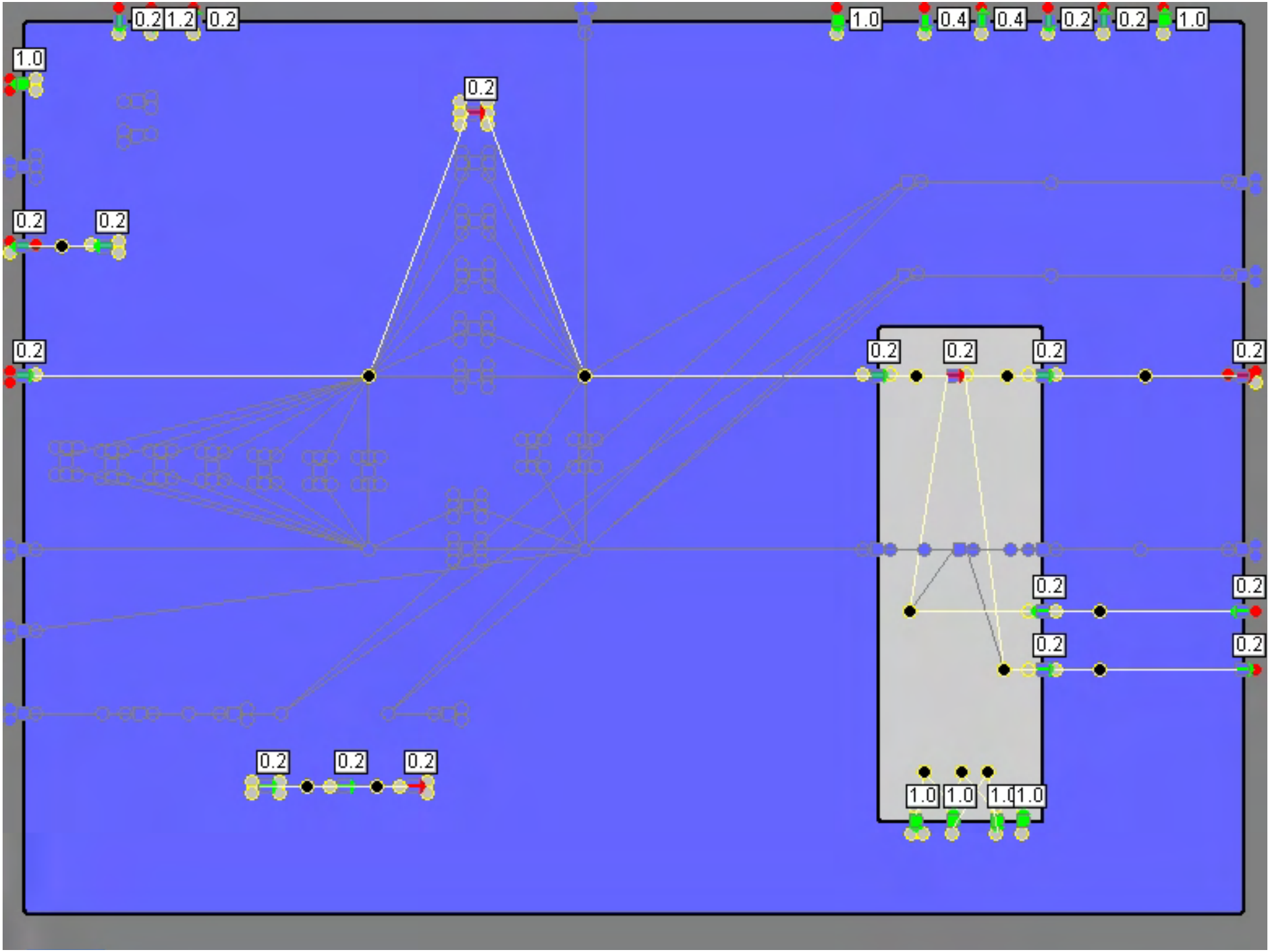
1.0
2.5
2.0
2.0
1.0
1.0
2.5
2.5
0.5
0.5
1.0
1.0
1.0
0.5
0.5
0.5
0.5
0.5
0.5
0.5
0.5
0.5
0.5
1.0
1.0
1.0


0.2
1.2
0.2
1.0
0.4
0.4
0.2
0.2
1.0
1.0
0.2
0.2
0.2
0.2
0.2
0.2
0.2
0.2
0.2
0.2
0.2
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0.2
0.2
0.2
0.2
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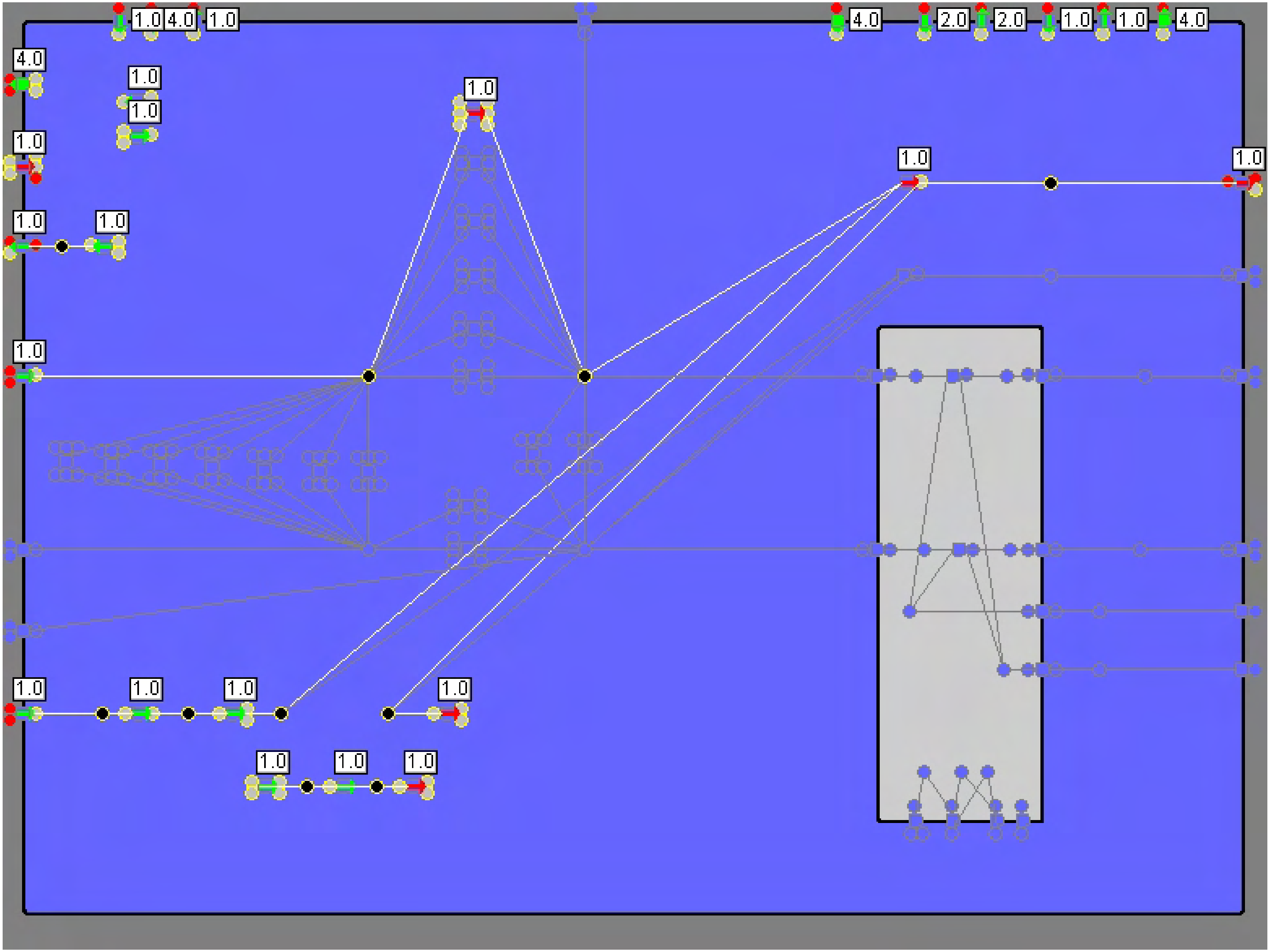
1.0
1.0
1.0



1.0
4.0
1.0
4.0
2.0
2.0
1.0
1.0
4.0
4.0
1.0
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1.0
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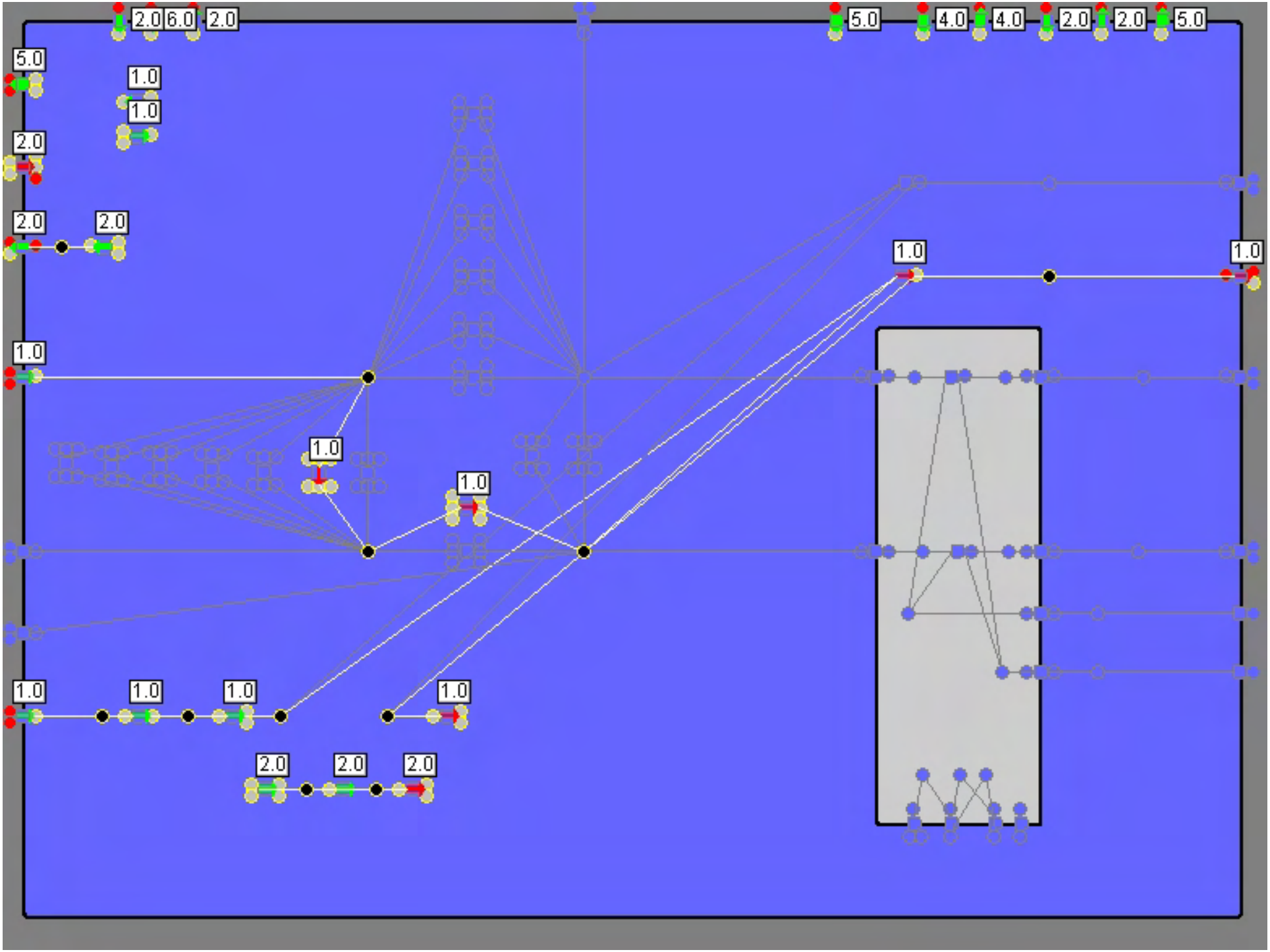
2.0
6.0
2.0
5.0
4.0
4.0
2.0
2.0
5.0
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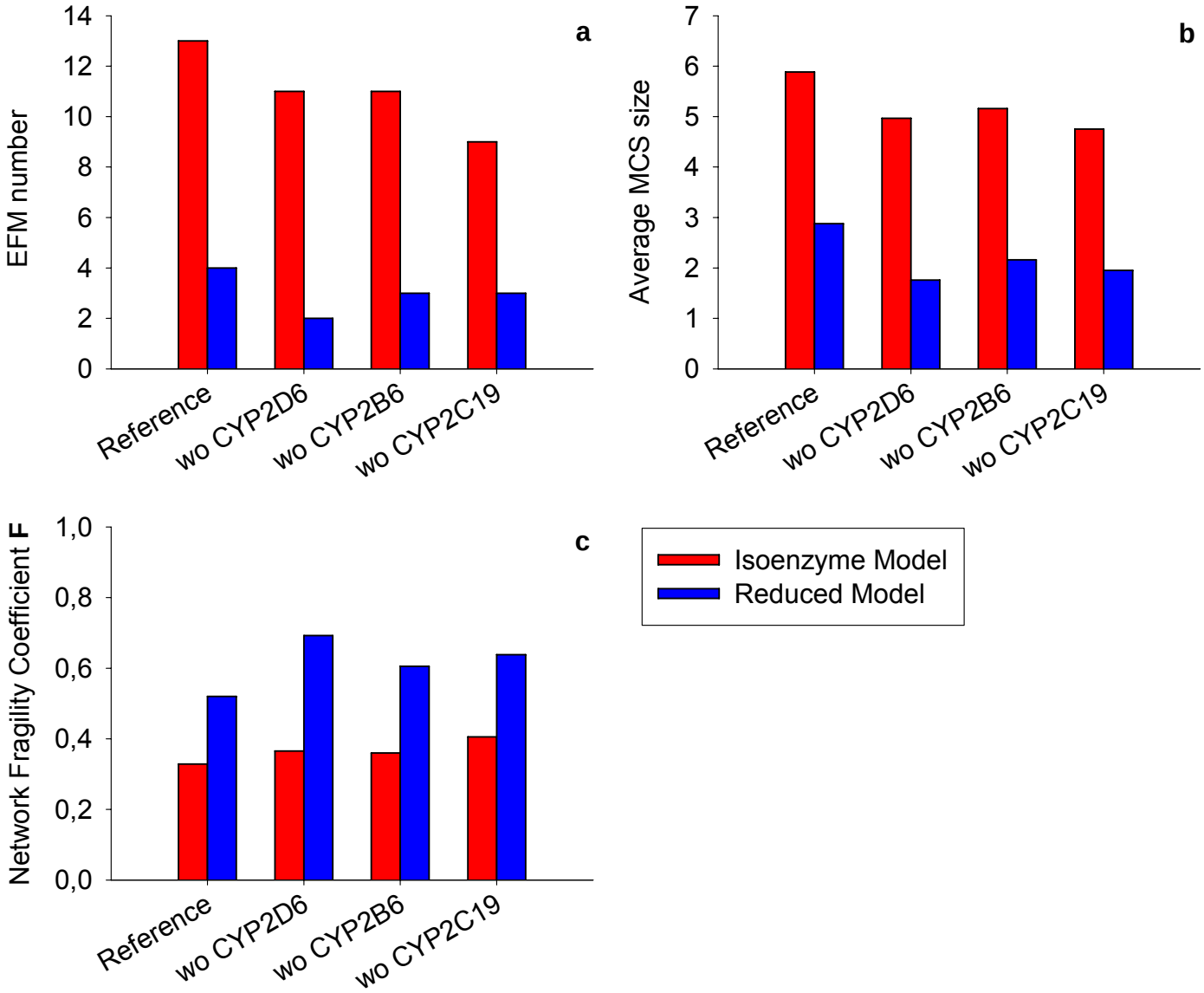
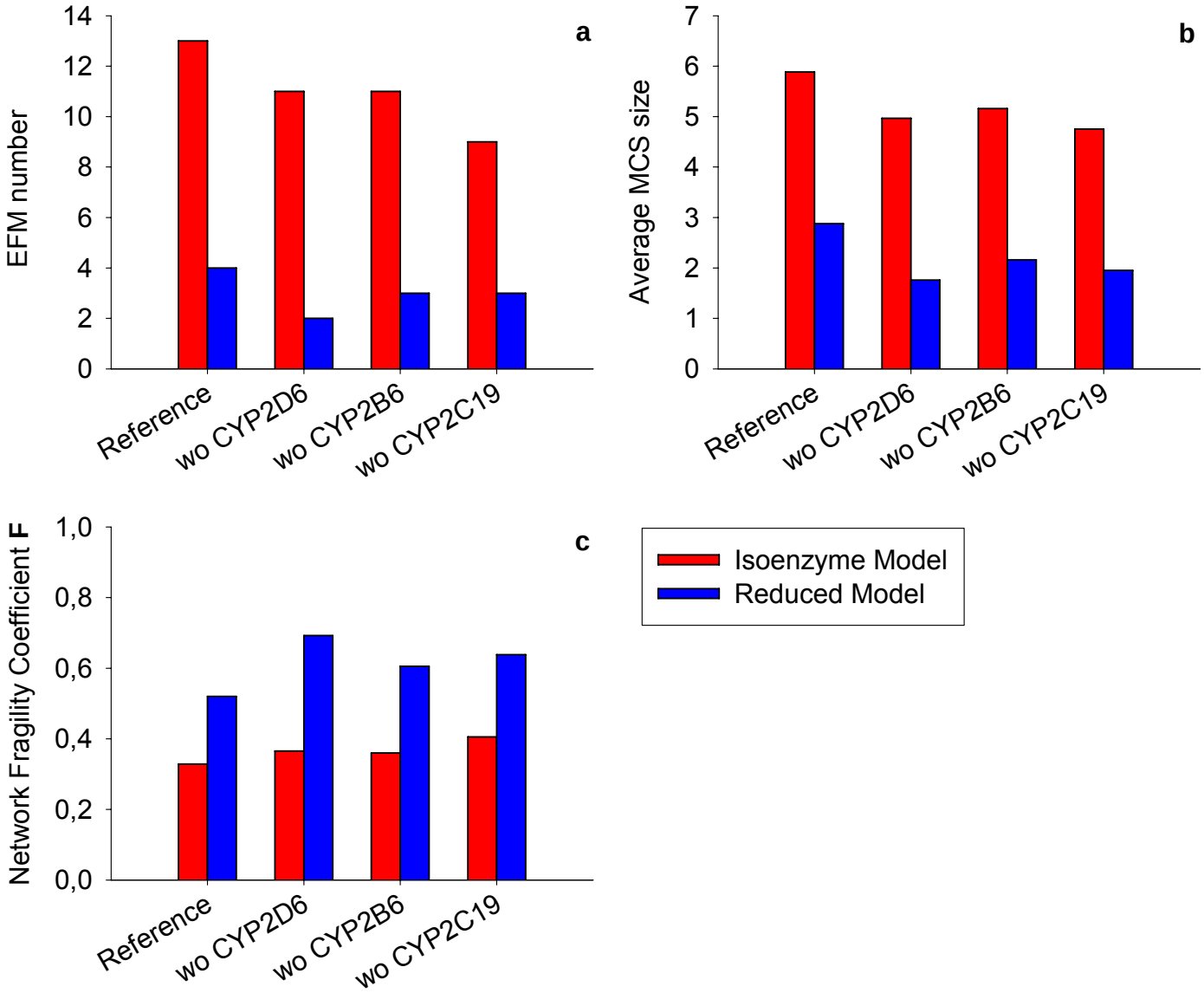
2.0


a
EFM number
14
12
10
8
6
4
2
0
Reference
wo CYP2D6
wo CYP2B6
wo CYP2C19
b
Average MCS size
7
6
5
4
3
2
1
0
Reference
wo CYP2D6
wo CYP2B6
wo CYP2C19
c
Network Fragility Coefficient F
1,0
0,8
0,6
0,4
0,2
0,0
Reference
wo CYP2D6
wo CYP2B6
wo CYP2C19
Isoenzyme Model
Reduced Model

MINIMAL CUT SETS (MCS)

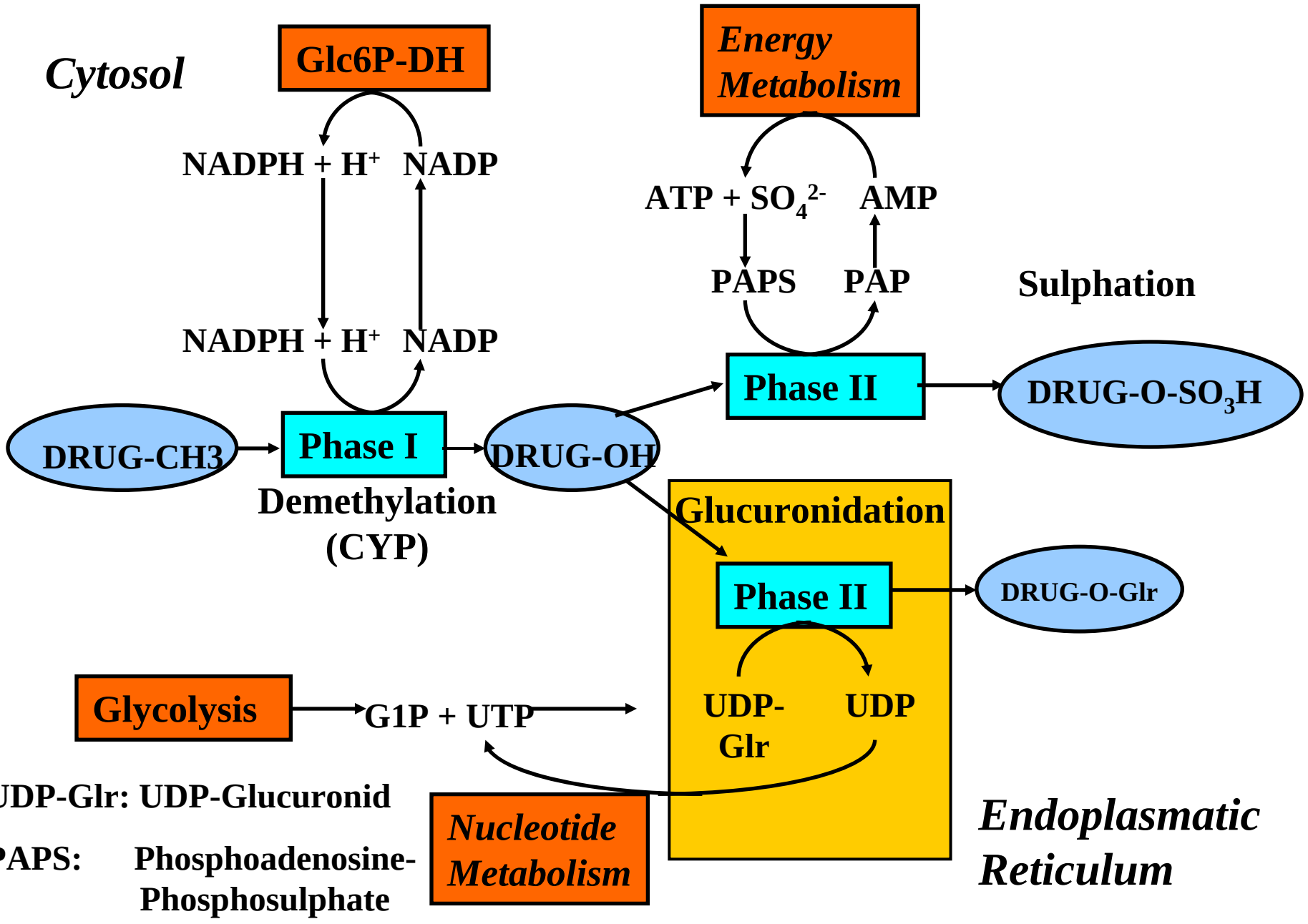
A MCS can be considered as a minimal set of events (loss of reactions) which – if these events occur together - leads to system failure, i.e. that the objective reactions cannot operate in a balanced fashion.

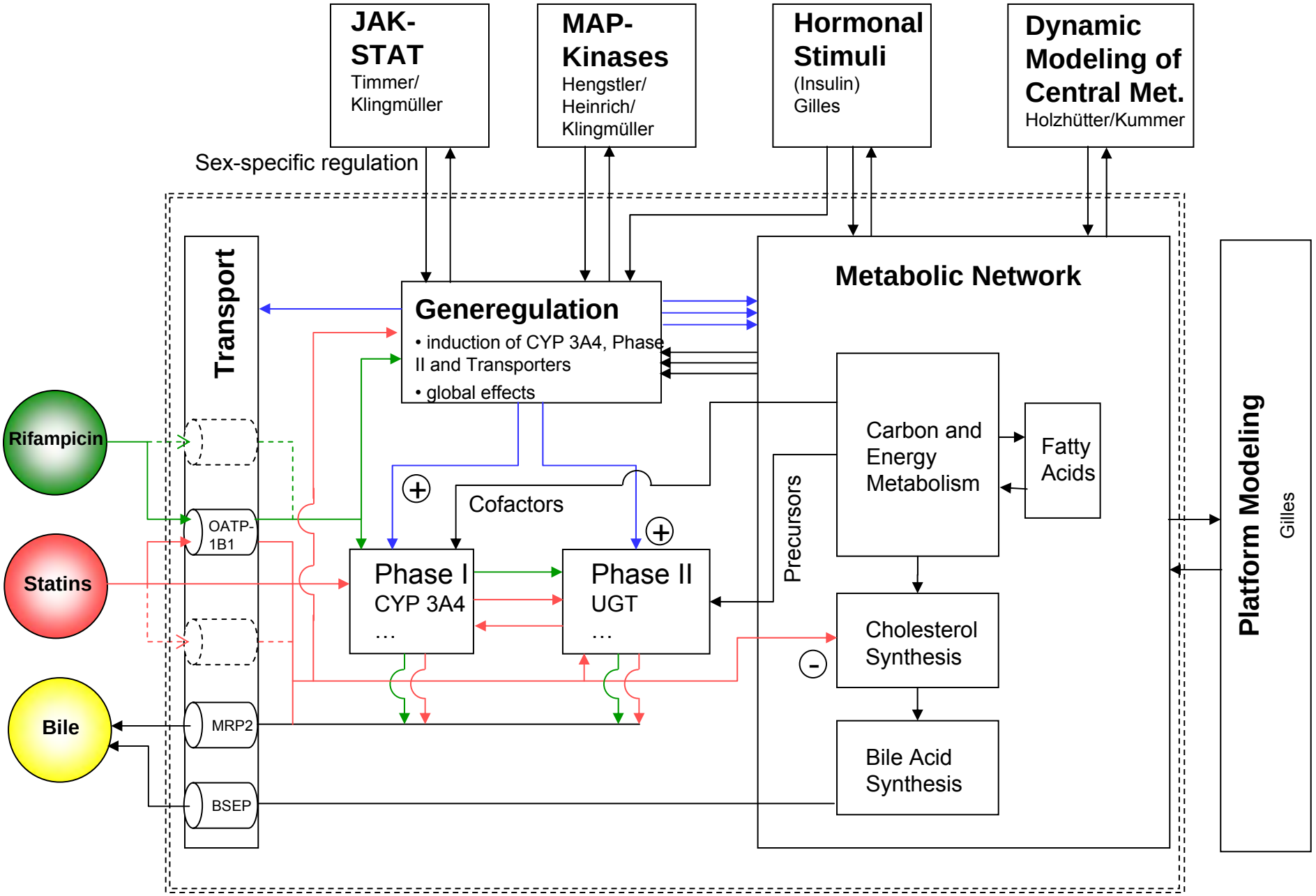

a
EFM number
14
12
10
8
6
4
2
0
Reference
wo CYP2D6
wo CYP2B6
wo CYP2C19
b
Average MCS size
7
6
5
4
3
2
1
0
Reference
wo CYP2D6
wo CYP2B6
wo CYP2C19
c
Network Fragility Coefficient F
1,0
0,8
0,6
0,4
0,2
0,0
Reference
wo CYP2D6
wo CYP2B6
wo CYP2C19
Isoenzyme Model
Reduced Model

Liver must be extremely robust because of the inter-individual isoenzyme variability (internal perturbations) and multitude of subtrates to be tackled by the P450 network (external perturbations)

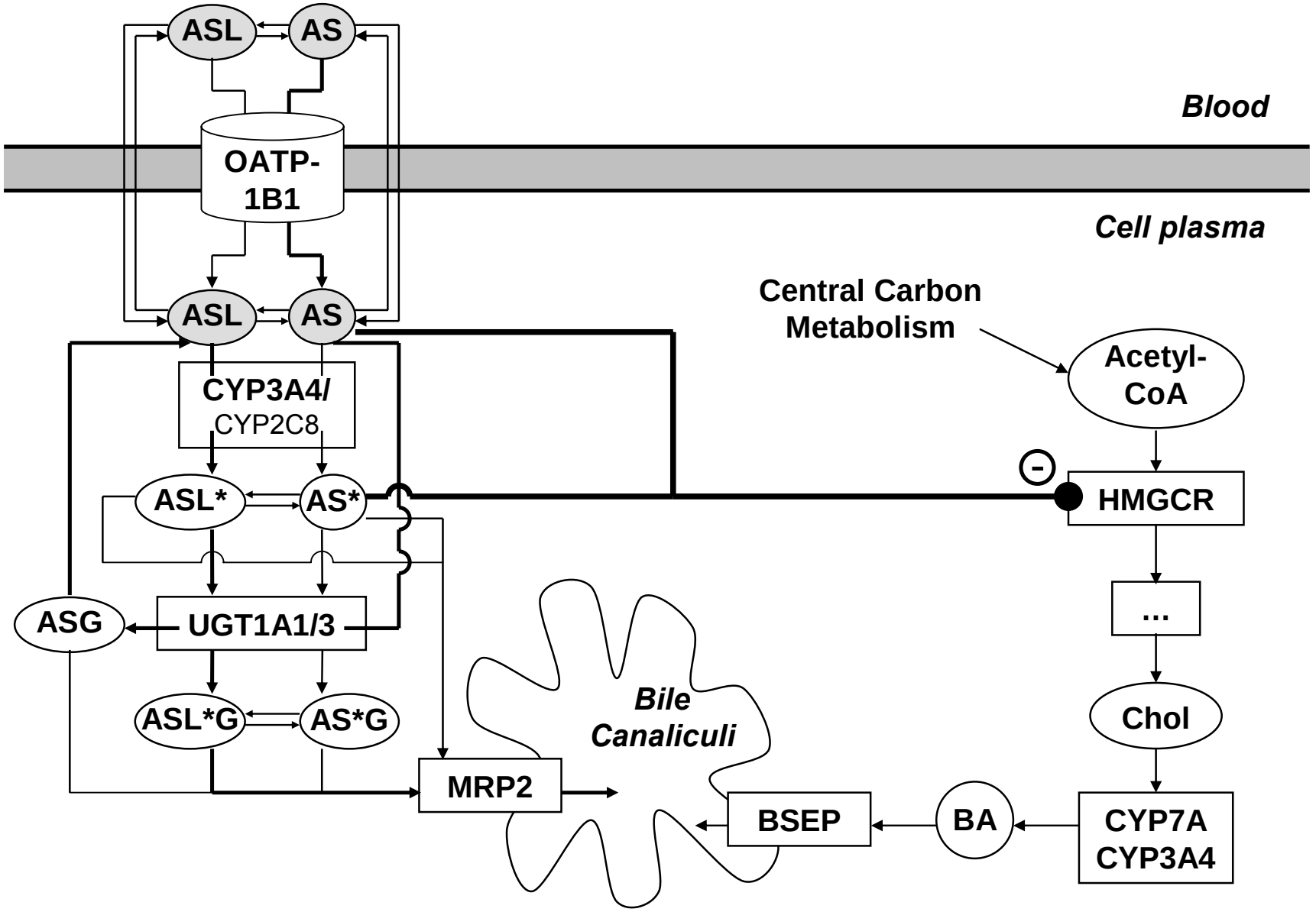
Stand-alone interpretation (kinetic modeling and simulation) of drug metabolism based on single isoenzyme activity (reductionistic pharmacokinetics) does not describe the system as a whole

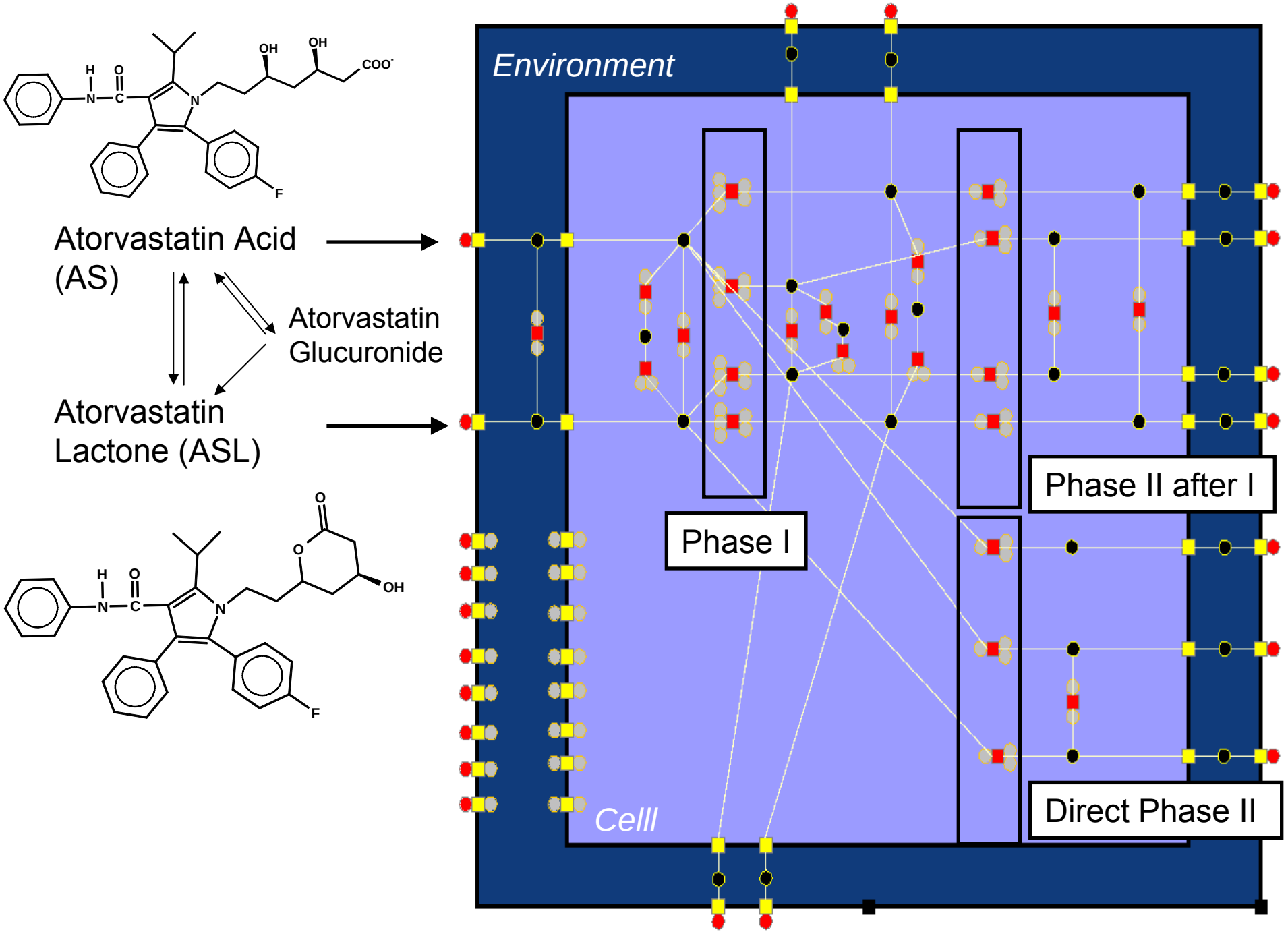
OUTLOOK –
ROAD MAP FOR FUTURE DIRECTIONS


Cytosol
Glc6P-DH
NADPH + H+
NADP
NADPH + H+
NADP
Energy Metabolism
ATP + SO4 2-
AMP
PAPS
PAP
Sulphation
Phase II
DRUG-O-SO3H
DRUG-CH3
Phase I
DRUG-OH
Demethylation (CYP)
Glucuronidation
Phase II
DRUG-O-Glr
Glycolysis
G1P + UTP
UDP-Glr
UDP
UDP-Glr: UDP-Glucuronid
PAPS: Phosphoadenosine-Phosphosulphate
Nucleotide Metabolism
Endoplasmatic Reticulum

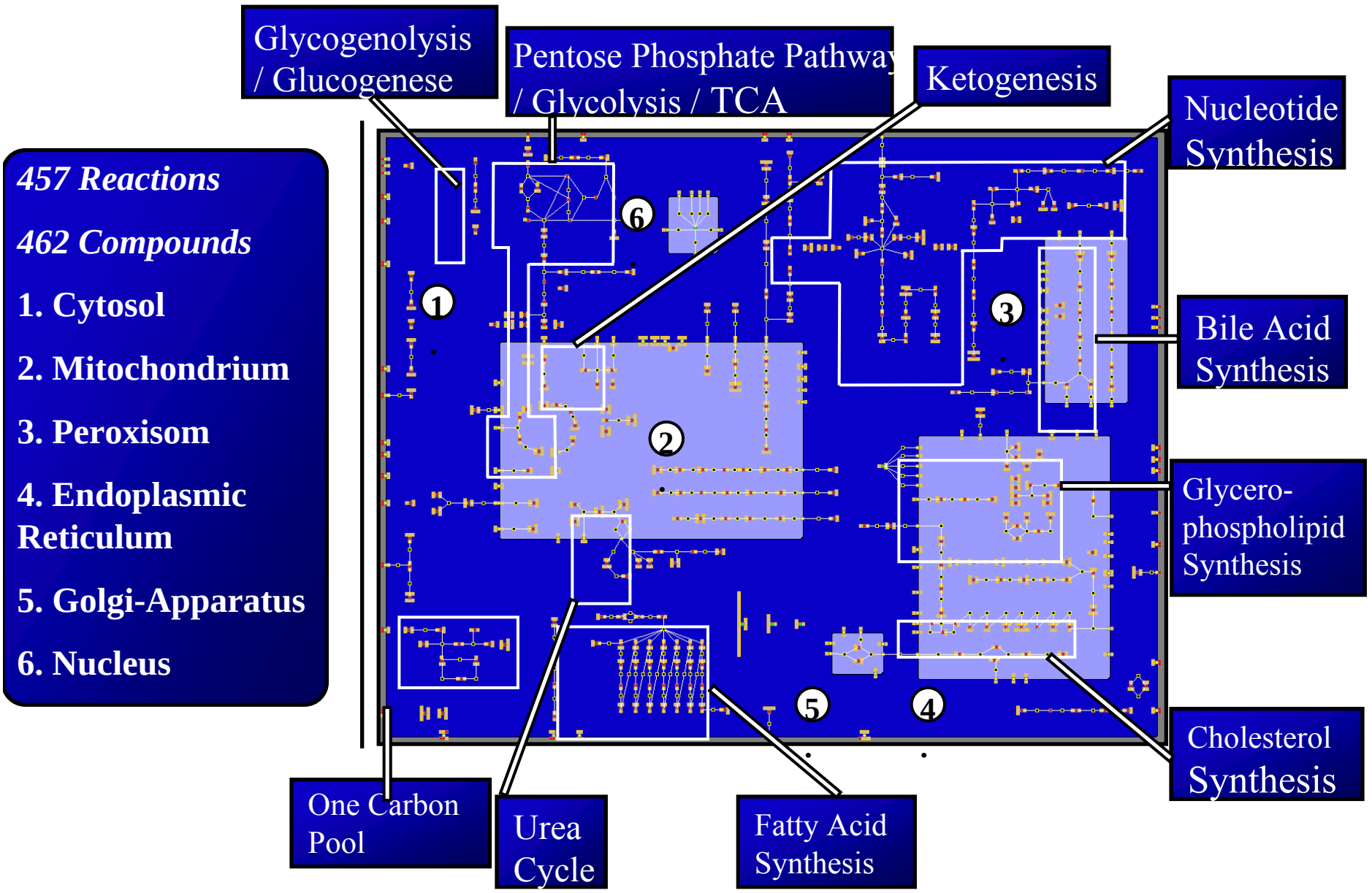

JAK-STAT
Timmer/ Klingmüller
MAP-Kinases
Hengstler/ Heinrich/ Klingmüller
Hormonal Stimuli
(Insulin)
Gilles
Dynamic Modeling of Central Met.
Holzhütter/Kummer
Sex-specific regulation
Transport
Generegulation
• induction of CYP 3A4, Phase II and Transporters
• global effects
Metabolic Network
Rifampicin
Statins
Bile
OATP-1B1
MRP2
BSEP
Cofactors
Phase I
CYP 3A4
…
Phase II
UGT
…
Precursors
Carbon and Energy Metabolism
Fatty Acids
Cholesterol Synthesis
Bile Acid Synthesis
Platform Modeling
Gilles

Atorvastatin (Sortis®, Lipitor®) (AS)

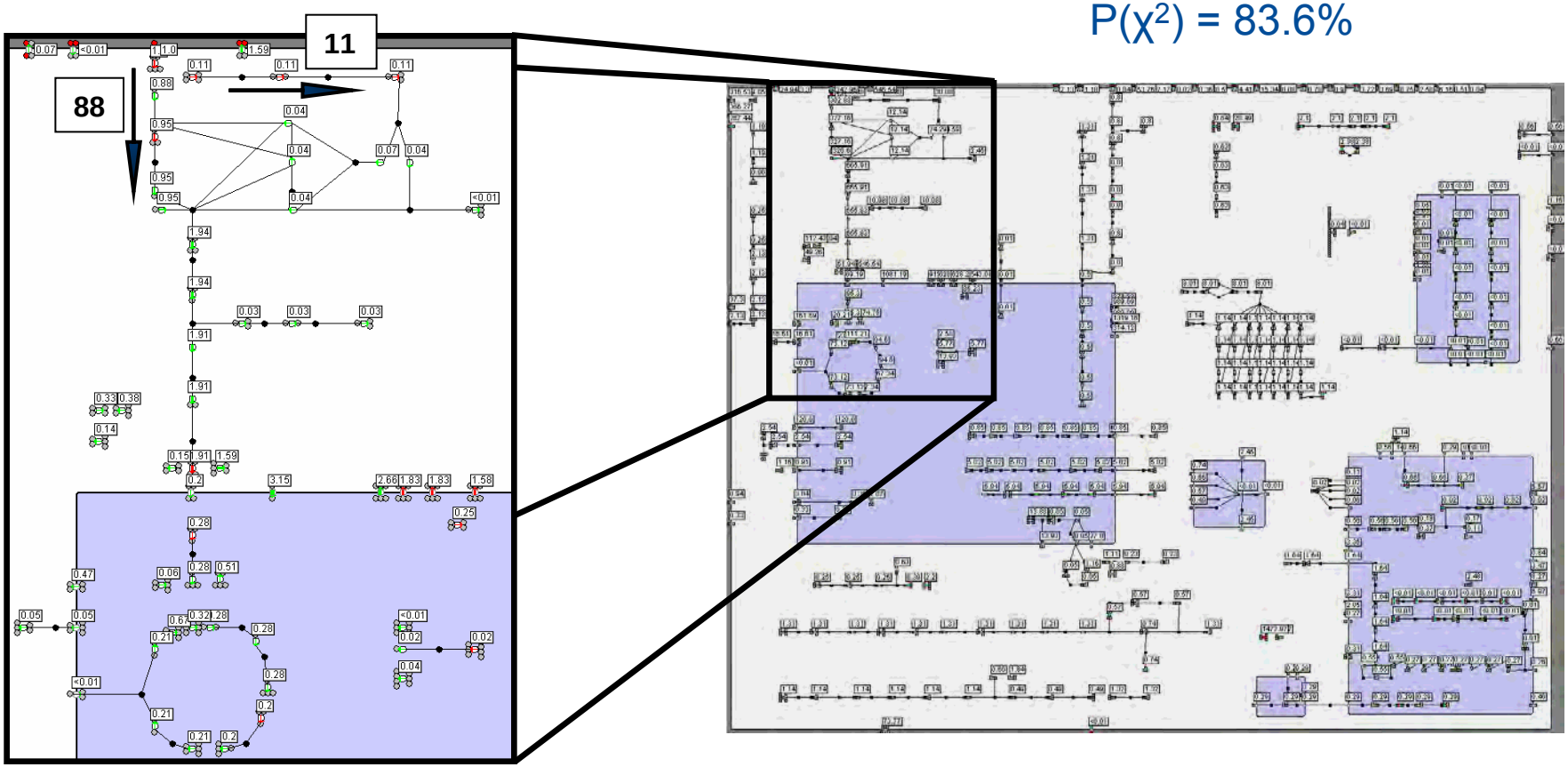



Atorvastatin Acid
(AS)
Atorvastatin
Glucuronide
Atorvastatin
Lactone (ASL)
Environment
Phase I
Phase II after I
Direct Phase II
CellI
Visualization with Insilico Discovery

Stoichiometric Model of the Hepatocyte

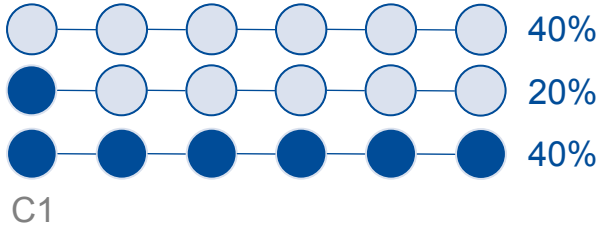


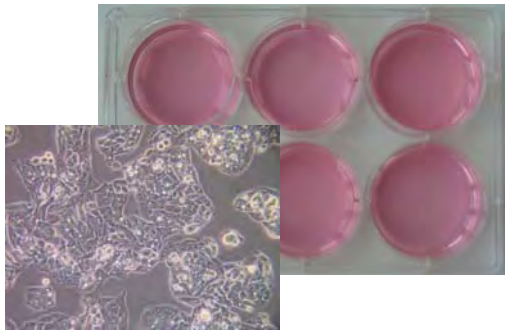
Metabolic Flux Analysis in HepG2



Labeling Experiment → **Flux Identification** → **Flux Distribution**

Addition of labeled substrate at time 0





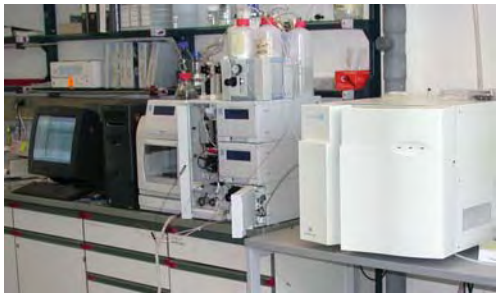
Hepatocytes “cultured“ in 6-well plates (-FCS, -Gln) 10^6 cells/ well

Quenching and cell disruption at various time points

Quantification by GC-MS and LC-MS at IKP

IKP STUTTGART

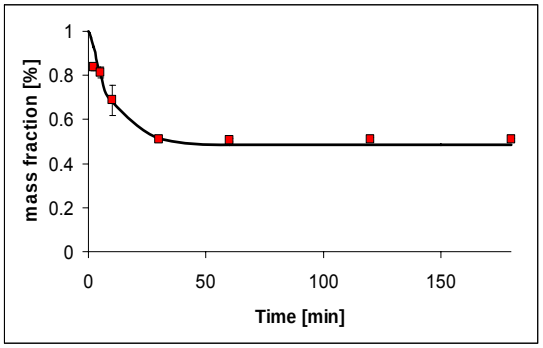
- 12 intracellular metabolite concentrations
- 67 mass isotopomers
- 26 extracellular metabolite concentrations

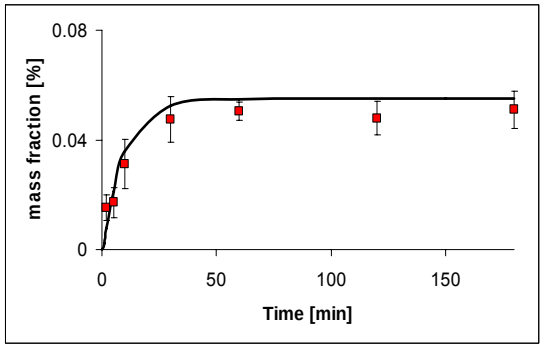


Glycolysis – 3-Phosphoglycerate

Experimental data — Model prediction

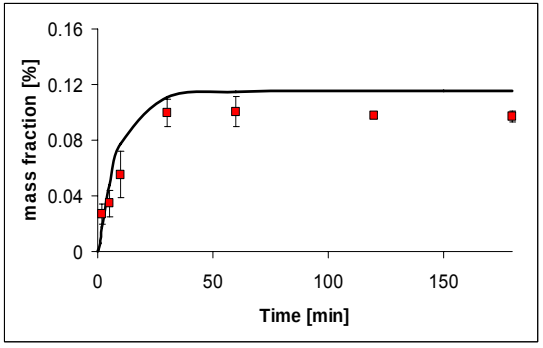
mo

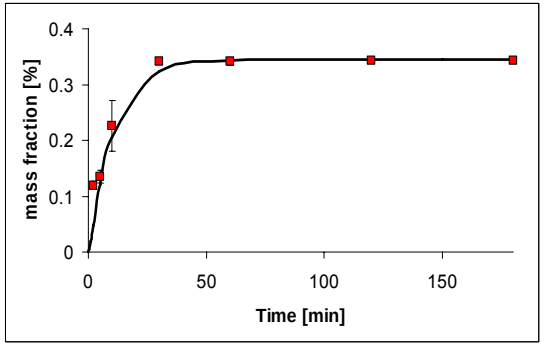




m2

m1

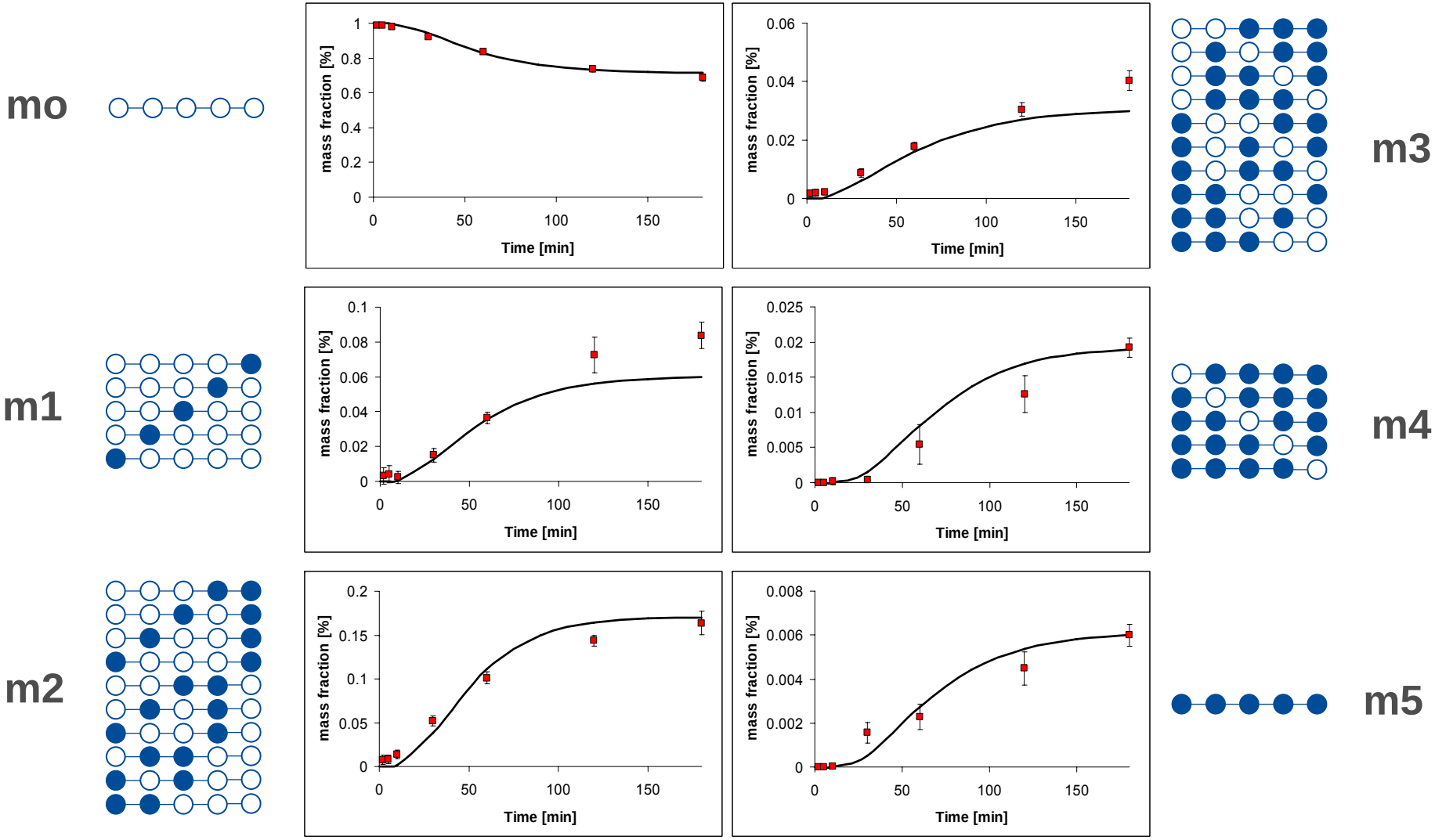




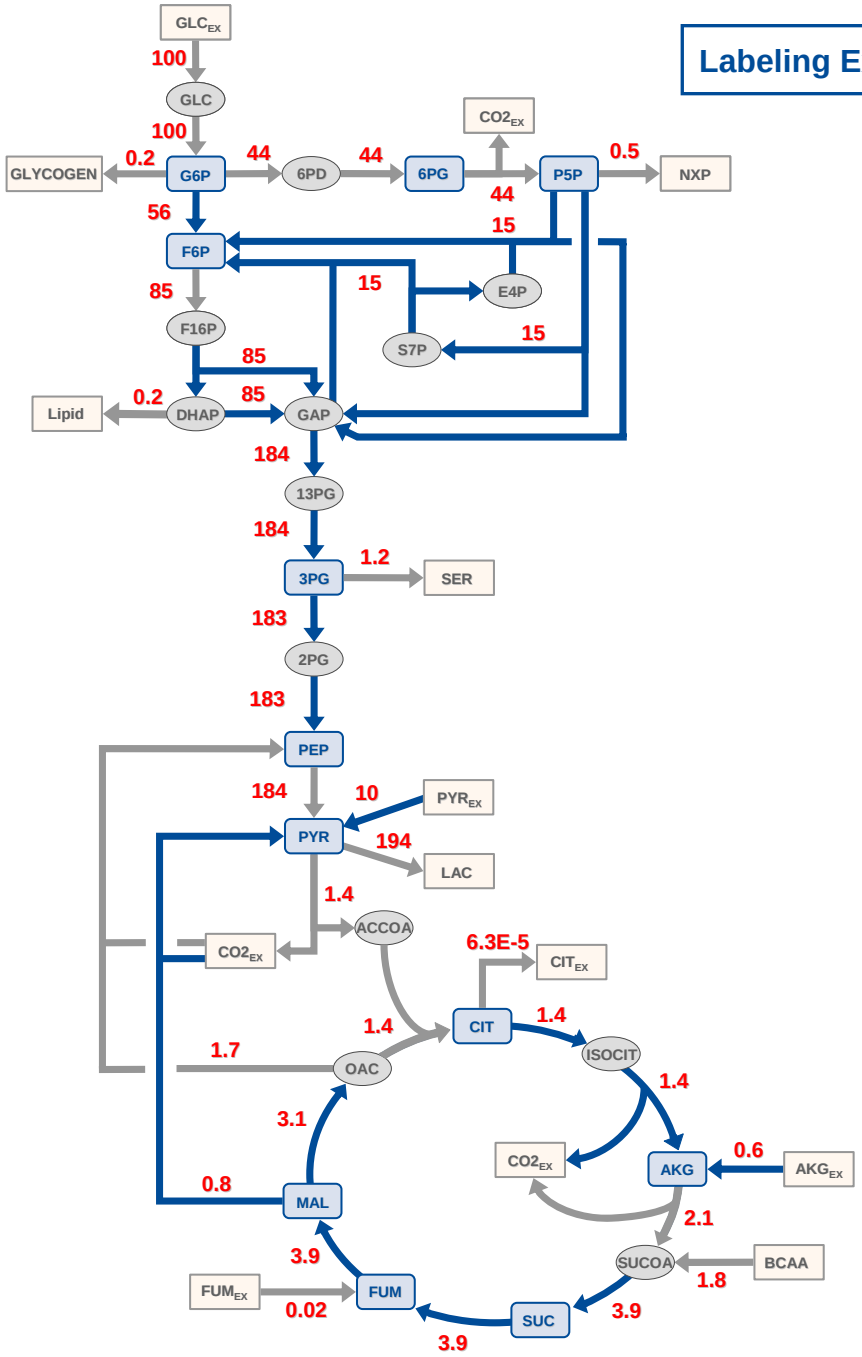
m3

TCA – α-Ketoglutarate

Experimental data — Model prediction



Labeling Experiment → Flux Identification → **Flux Distribution**



Split Ratio: Glycolysis - PPP

- *Metabolite Balancing*
  - 11% PPP; 88% Glycolysis
  - 5% PPP; 95% Glycolysis
- *Isotopomer Balancing*
  - 44% PPP; 56% Glycolysis

≡ Irreversible flux

≡ Reversible flux

≡ Balanced metabolite (Estimated pool concentration)

≡ Balanced metabolite (experimentally determined pool concentration)

≡ External metabolite

(1) Dynamics of gene regulation of the detoxification system

(Reverse engineering: Unraveling gene-gene interactions from Microarray data)

(2) Quantitative kinetics of drug induced interactions in the nuclear receptor network

(3) Chemoinformatics modeling
molecular modeling: from sequence to function

(4) High throughput data: microarrays and proteomics

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(5) Hierarchical multi scale modeling:

Integration of single hepatocyte models with whole body (multi organe) models

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Klaus Maier
Klaus Mauch

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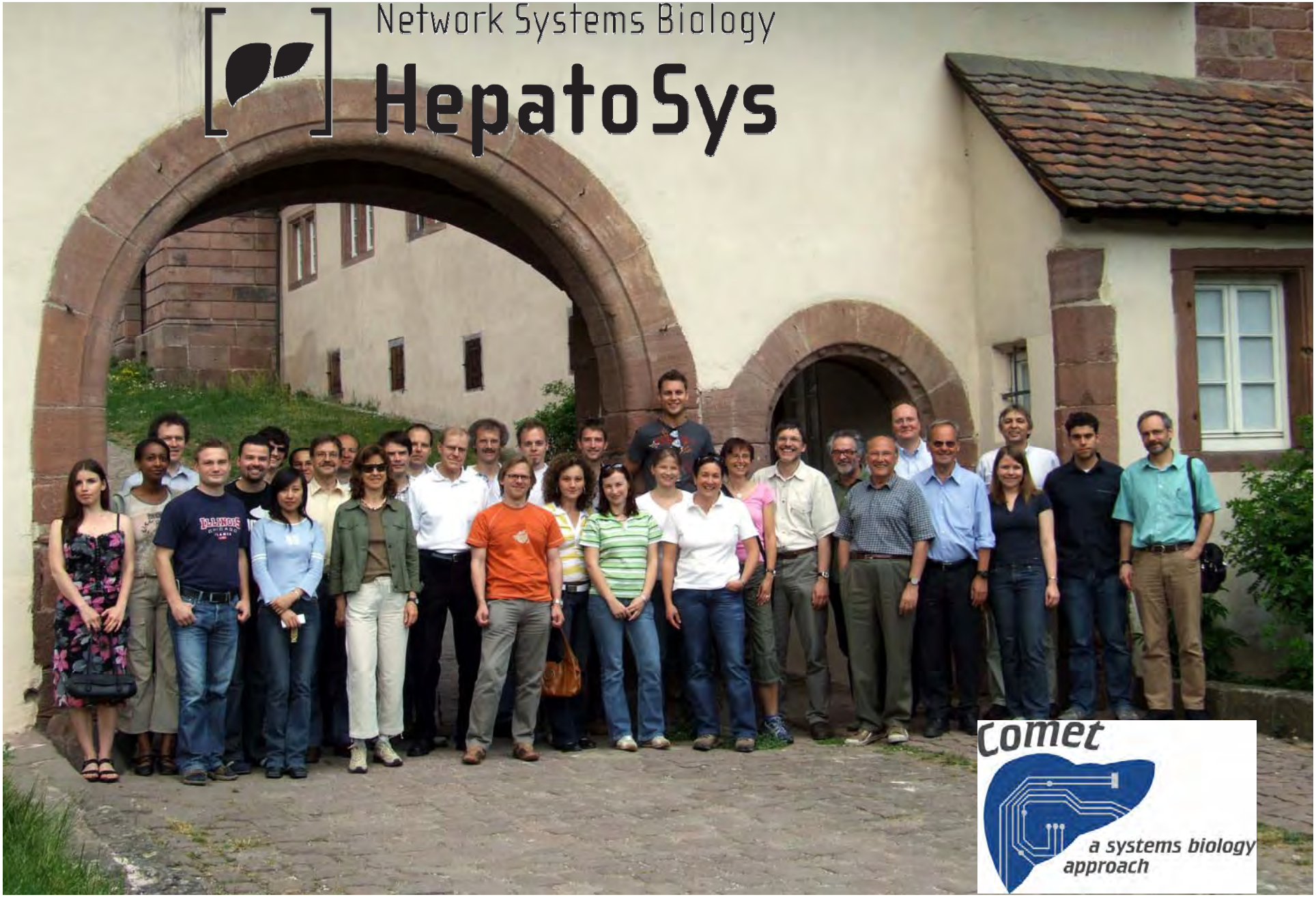
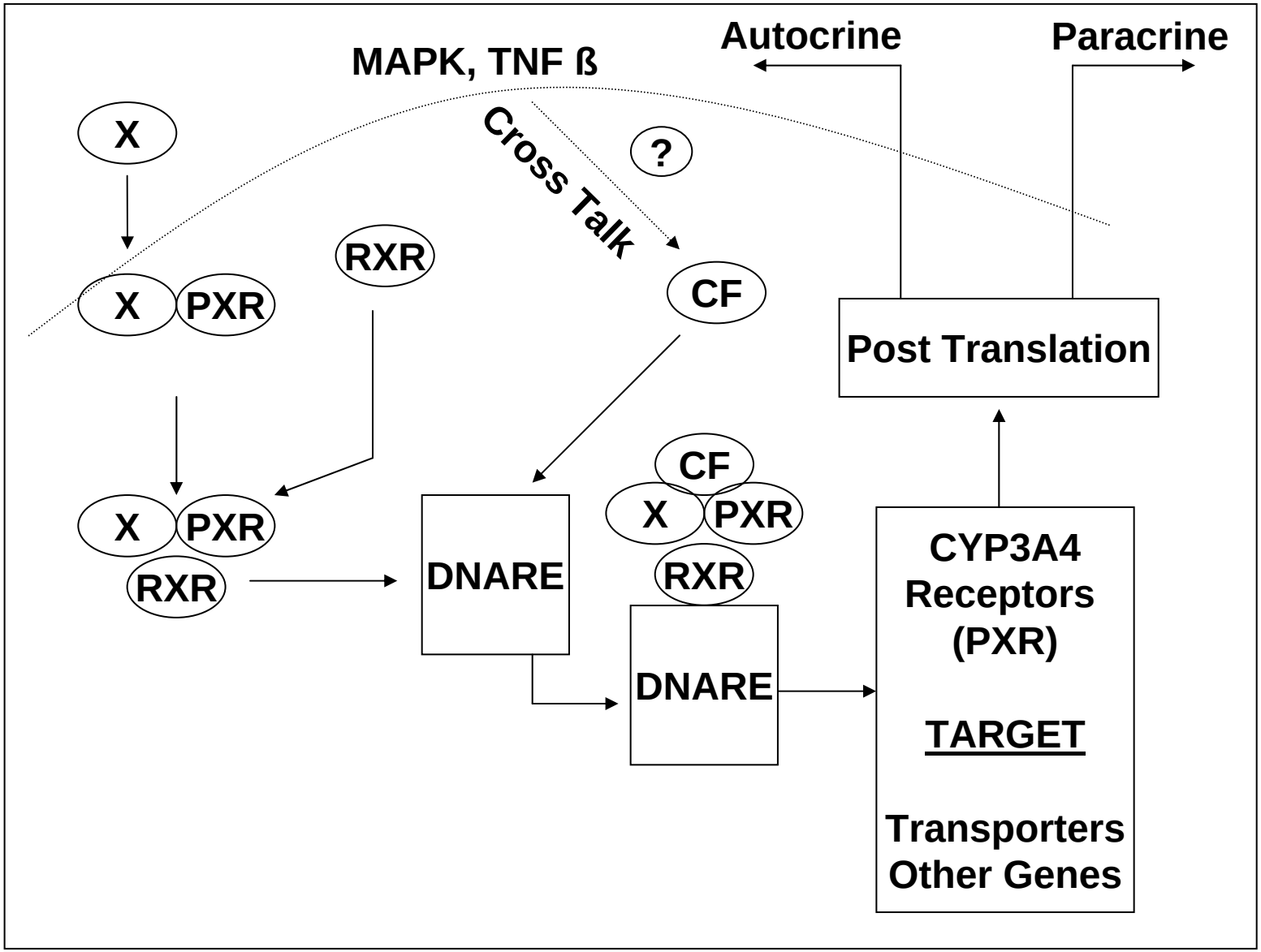
Bundesministerium
für Bildung
und Forschung

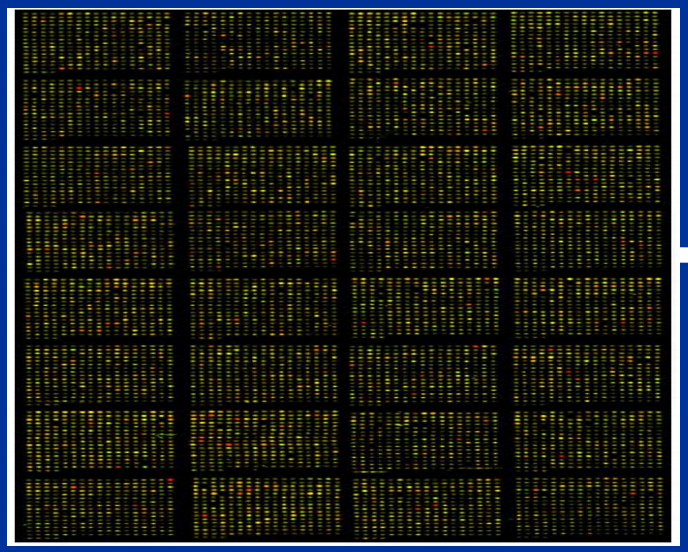


Network Detoxification

Phase I Module



Data Analysis - Work Flow



Array Data (Time Series)

Cluster Analysis

Normalization Calculation of the mean and sd Coefficient of Variation

Sorting

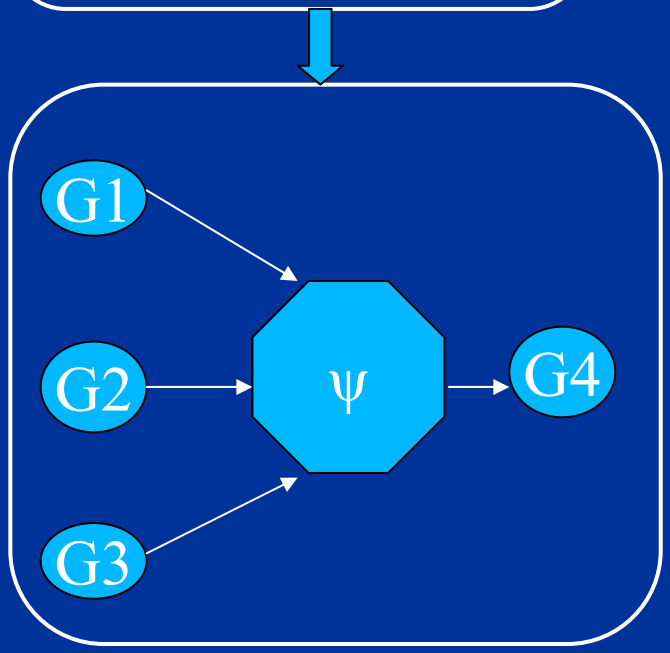
Discretisation (1,0,-1) Predictor Gene Sets

Influence

Target Genes (Problem Specific)

Methodology

Data Analysis → Predictor Combinations



Ψ = Predictor Function

$\theta = [\varepsilon(\Psi_0) - \epsilon(\Psi_{opt})] / \epsilon(\Psi_0)$

$\epsilon(\Psi)$ – Error Function

θ – Coefficient of Determination

$\theta > \theta_{treshold}$ -> prominent predictor set

CYP3A4 specific Predictors

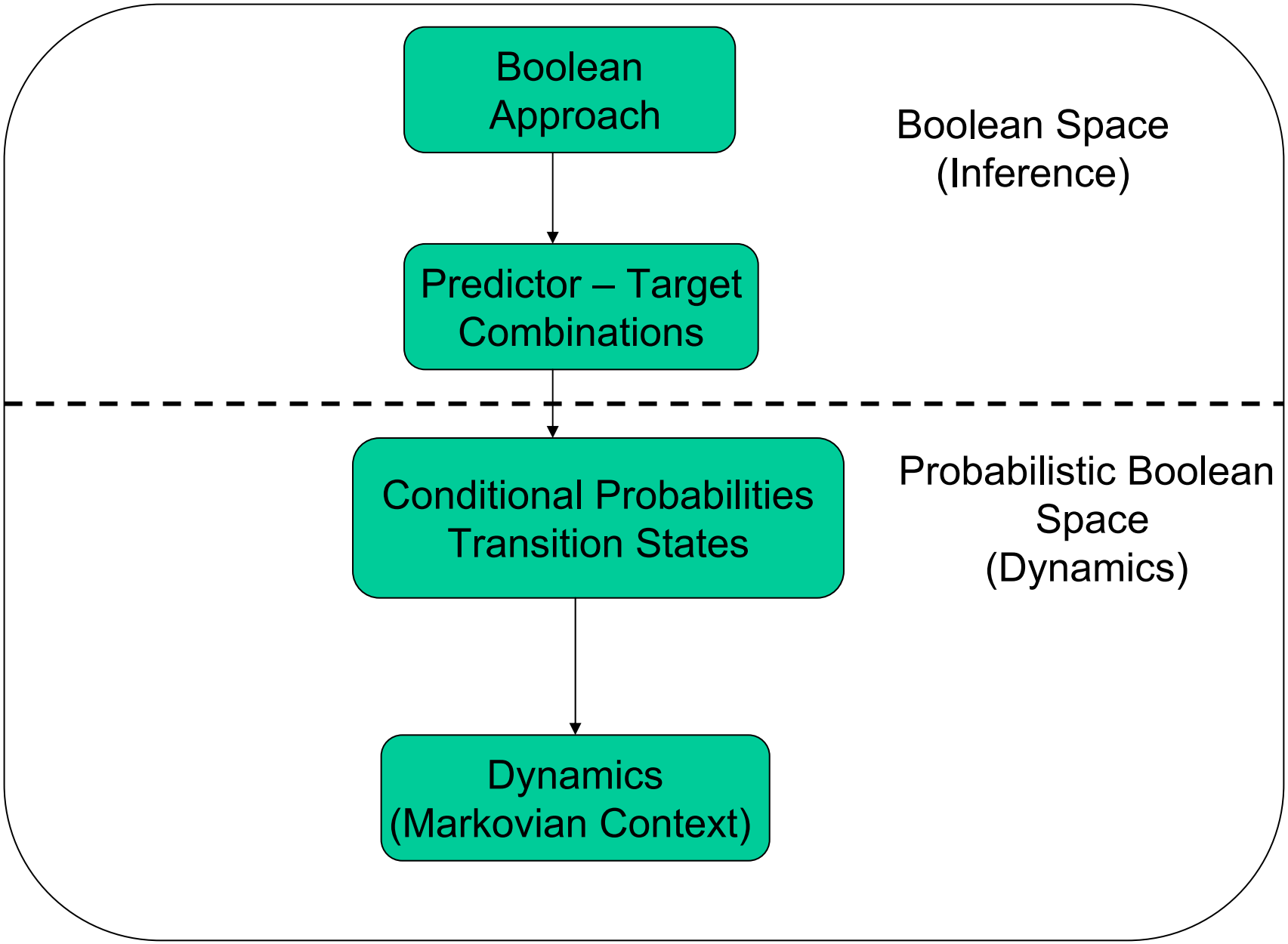
Most Prominent Predictors for CYP3A4 – 37

Predictor	Percentage
PXR	21
MAPK3	19
G6PT	16
CDC14B3	16
RXR	14
HNF 4 – alpha	14
ERK1/2	12
MDR1	9
PRO0786	9
GST2	7
OATP2	5

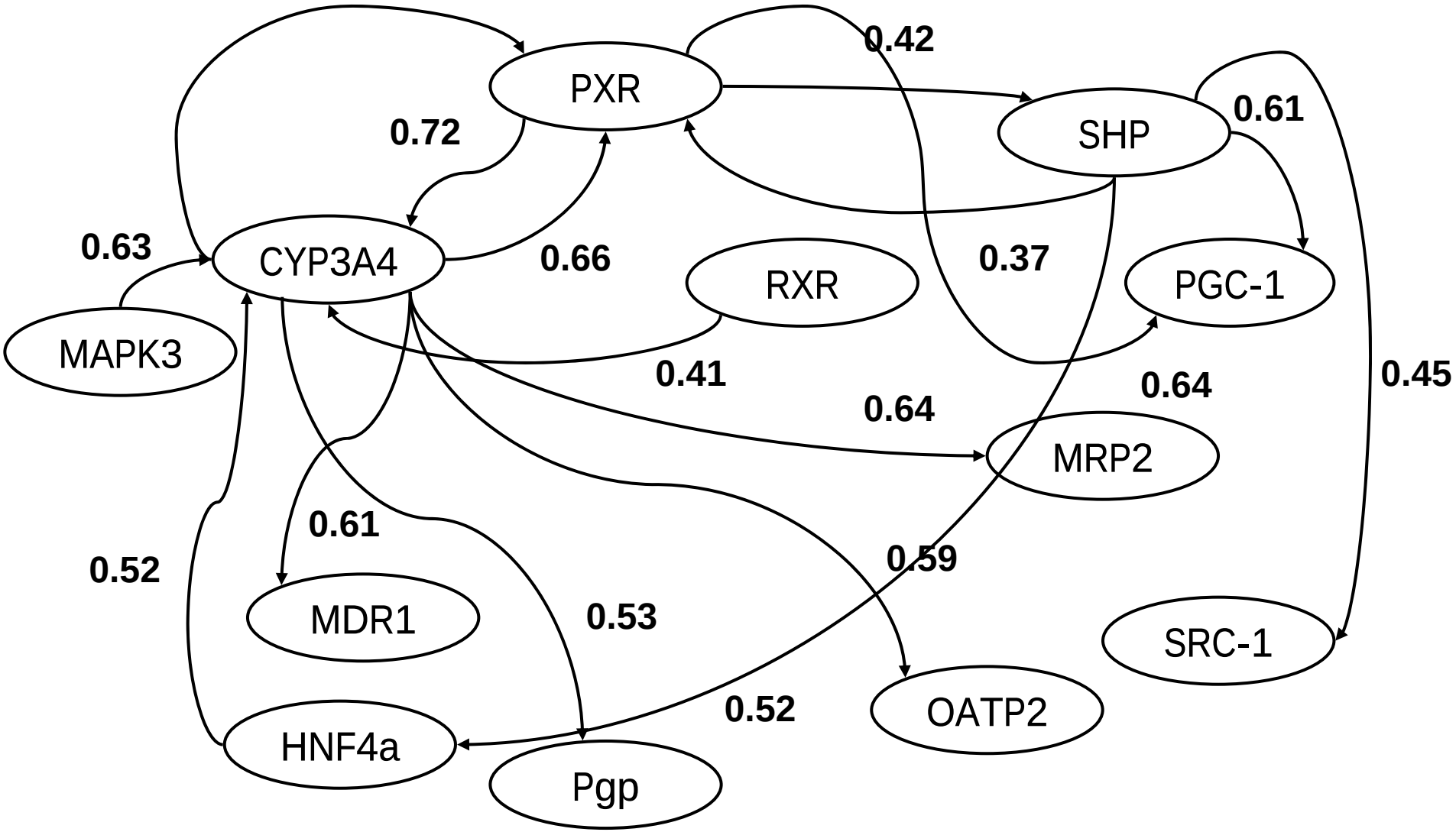
% = No. of times a given predictor was used for a given target

(p48 protein)

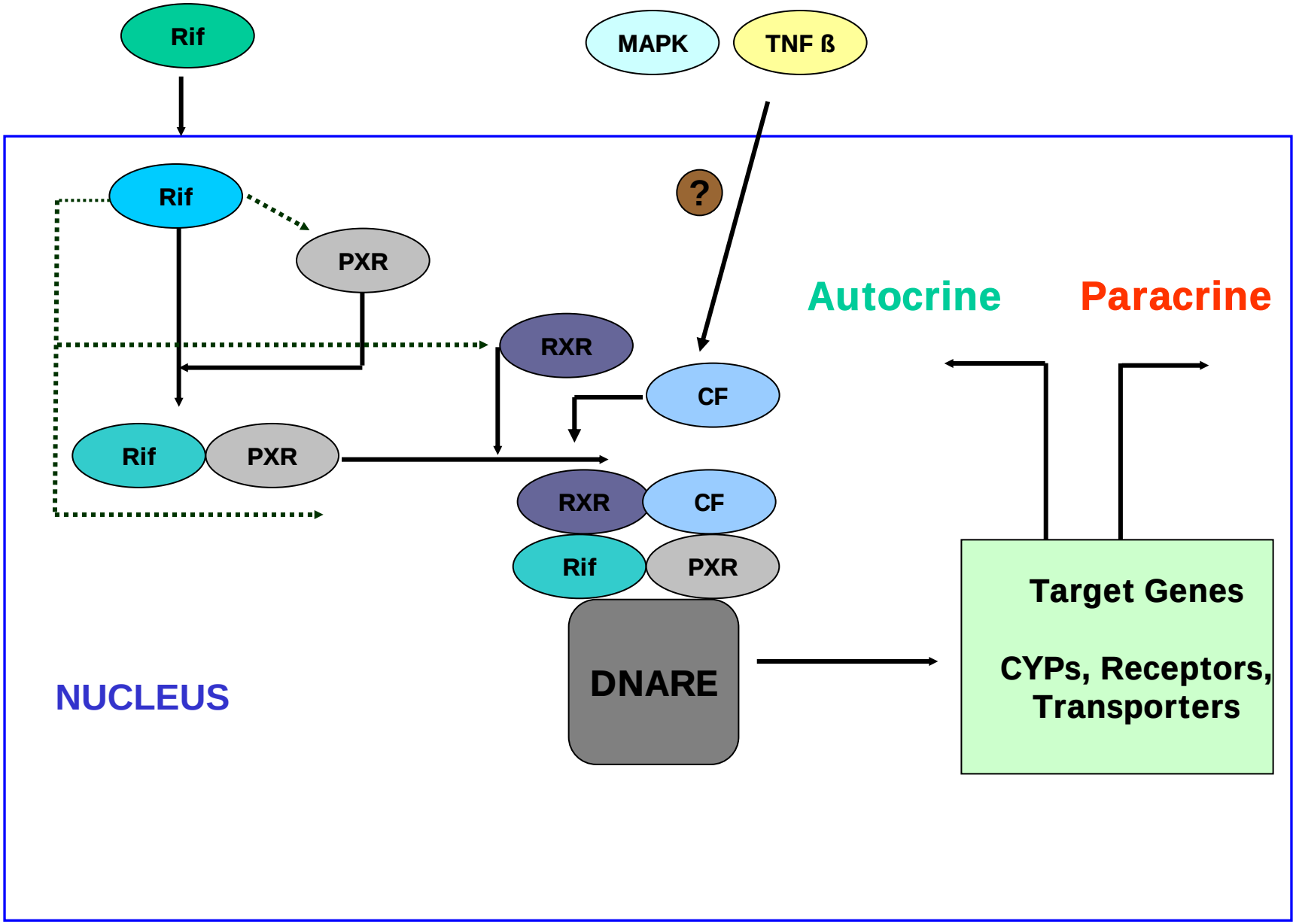
Boolean to Probabilistic Boolean



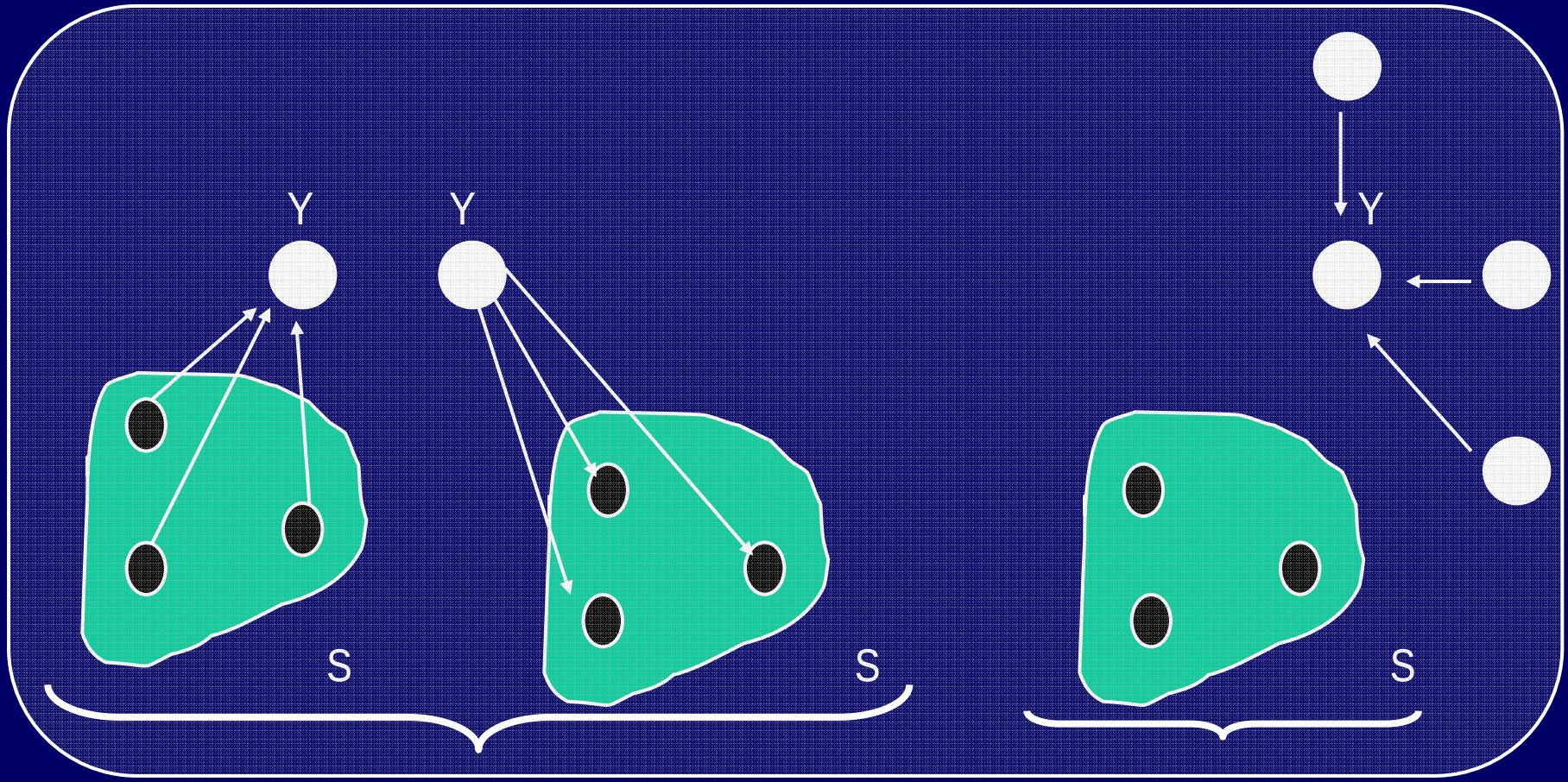
Subnetwork Simulation



0.XX – Connection Strength (Probabilities)

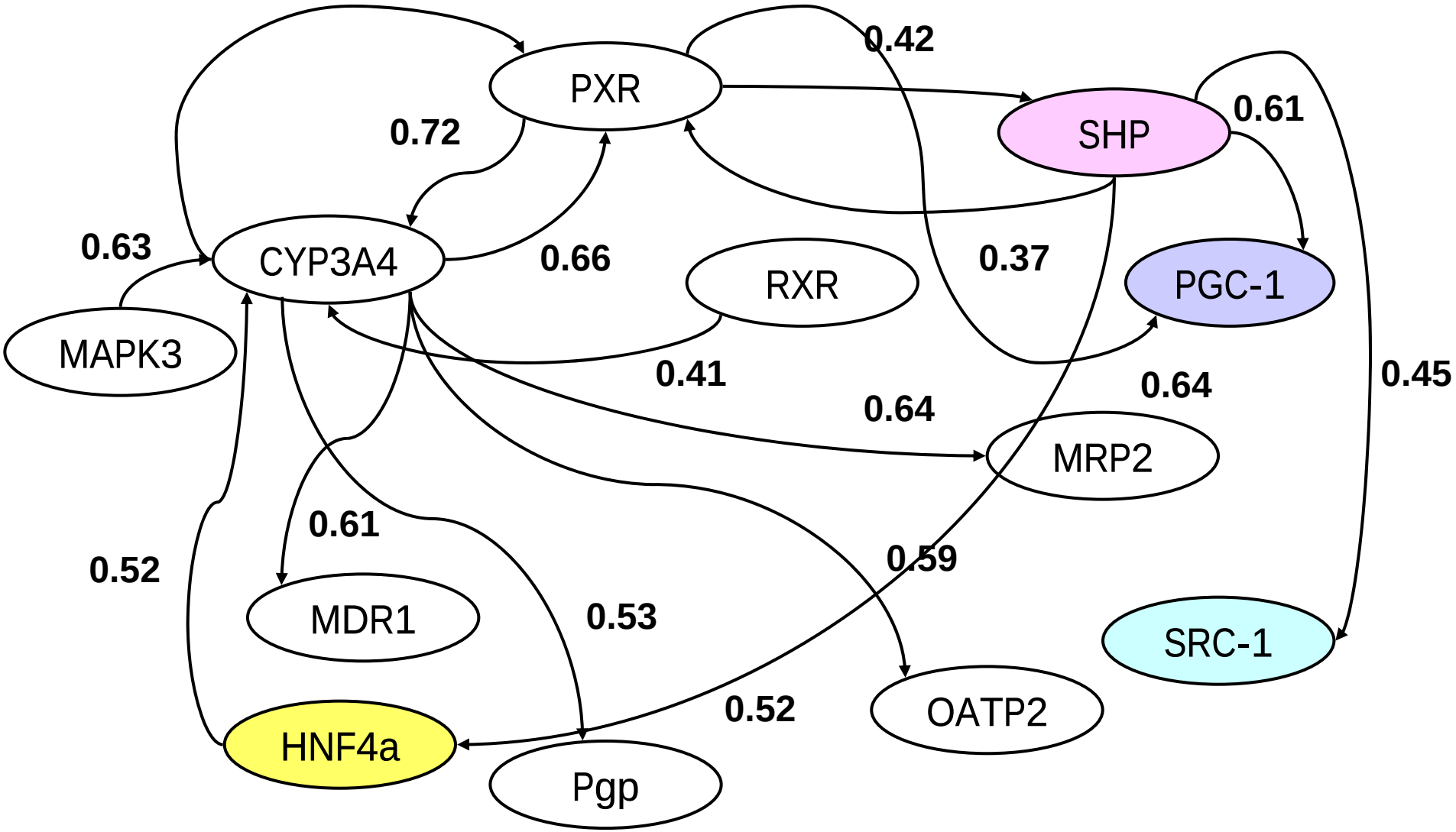

Rif
MAPK
TNF ß
Rif
?
PXR
Autocrine
Paracrine
RXR
CF
Rif
PXR
RXR
CF
Rif
PXR
DNARE
Target Genes
CYPs, Receptors,
Transporters
NUCLEUS

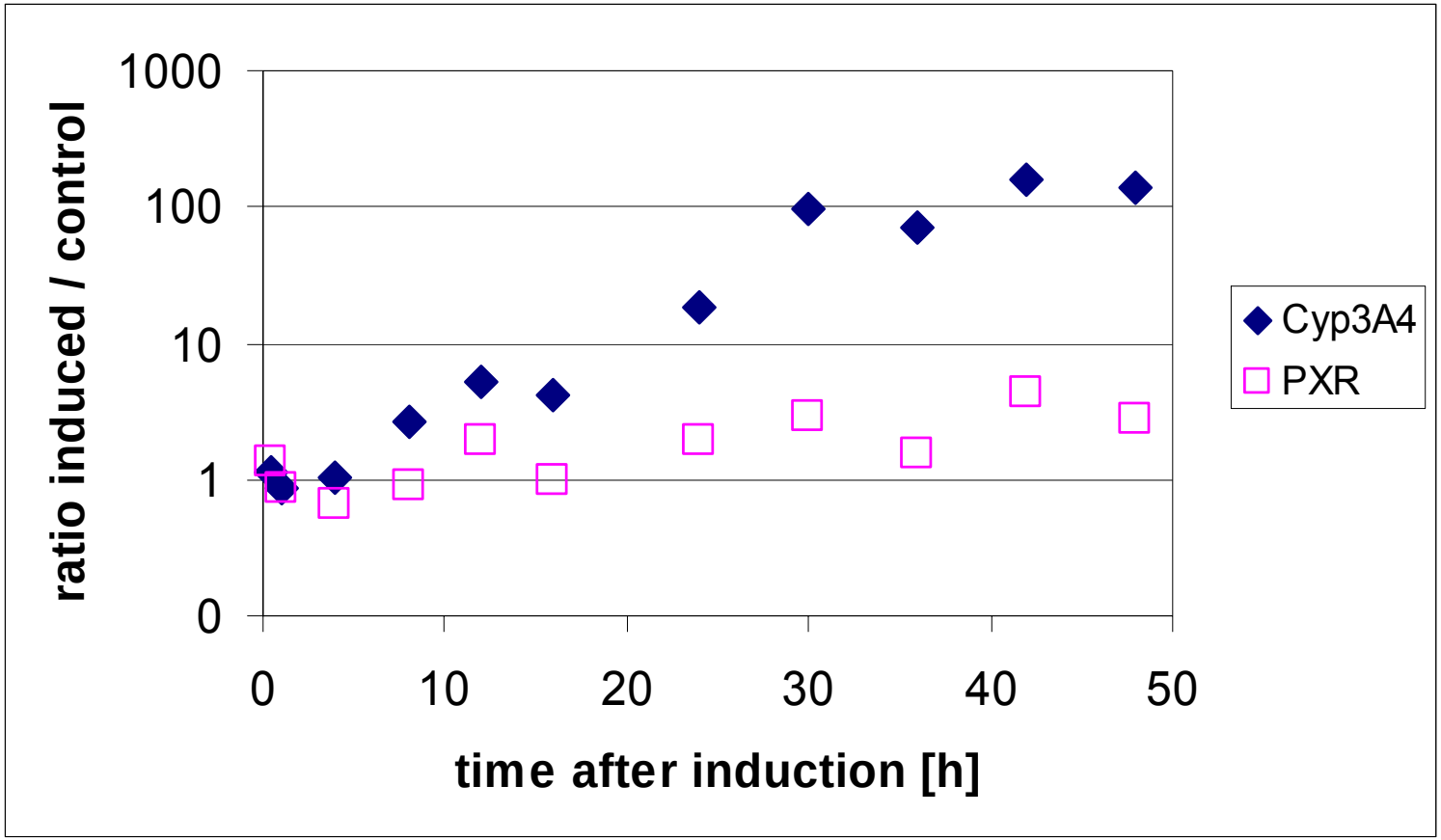
Principle of Autonomy



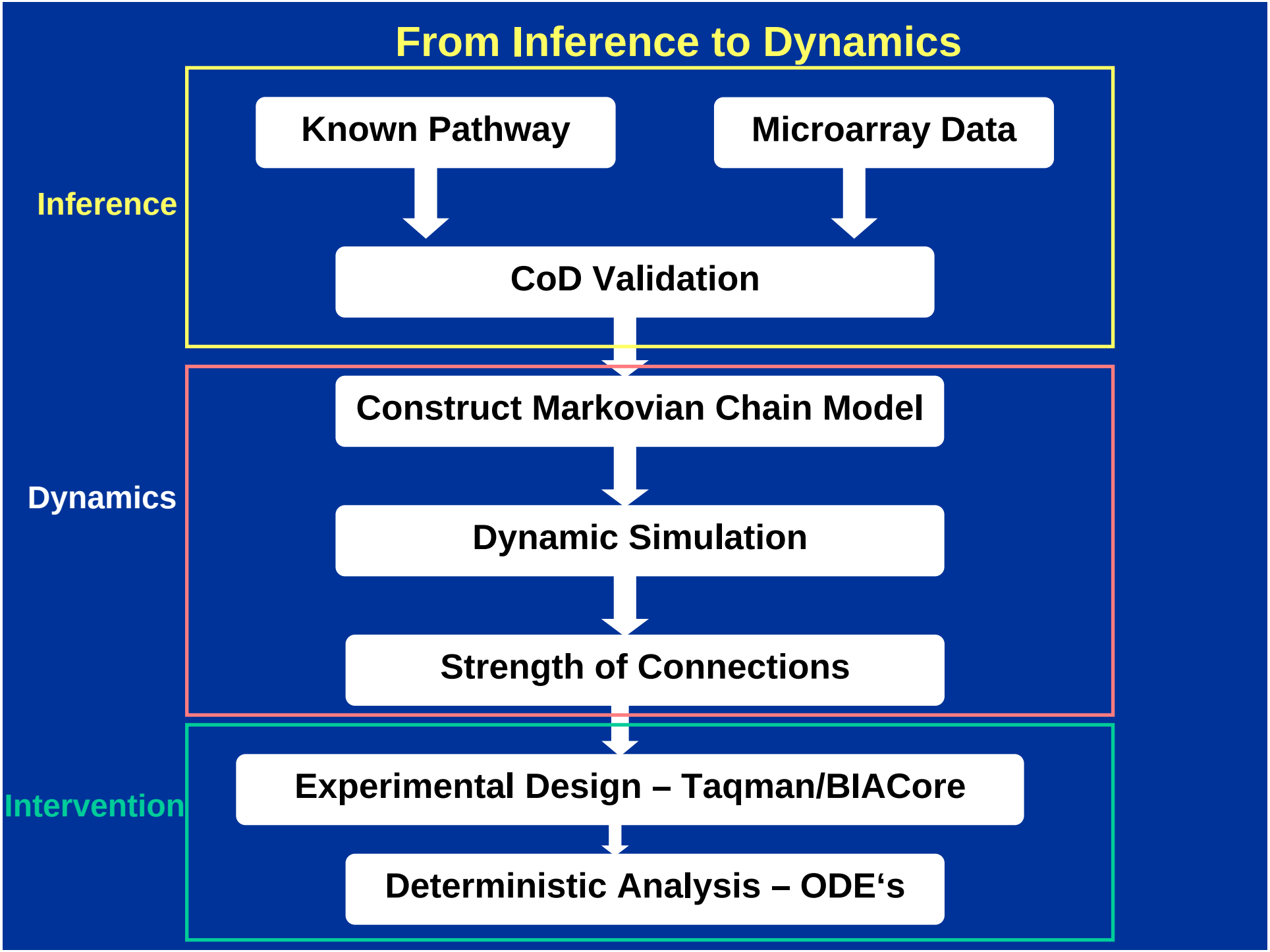
To achieve network autonomy, both of these strengths of connections should be high

The sensitivity of Y from the outside should be small


PXR
SHP
CYP3A4
RXR
PGC-1
MAPK3
MRP2
MDR1
SRC-1
OATP2
HNF4a
Pgp
0.42
0.72
0.61
0.63
0.66
0.37
0.41
0.64
0.64
0.45
0.61
0.52
0.59
0.53
0.52
0.XX – Connection Strength (Probabilities)



Thomas Reichart, ITB, Uni Stuttgart


From Inference to Dynamics
Inference
Known Pathway
Microarray Data
CoD Validation
Dynamics
Construct Markovian Chain Model
Dynamic Simulation
Strength of Connections
Intervention
Experimental Design – Taqman/BIACore
Deterministic Analysis – ODE‘s

* from reverse (inverse) engineering to determinsitic modeling

- * boolean modeling – probalistic- boolean modeling - deterministic modeling

- * top-down/bottom-up/middle - out