virtual liver
network

Microarrays as a screening resource for multiscale models

Assessment of metabolic functions

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COMPUTATIONAL SYSTEMS BIOCHEMISTRY

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UNIVERSITÄTSMEDIZIN BERLIN

Outline

- Modeling aims in Virtual Liver
- ModeScore method
- TGFβ effects

- HGFα/IL6 effects

- Nuclear transcription factors

- Statin effects

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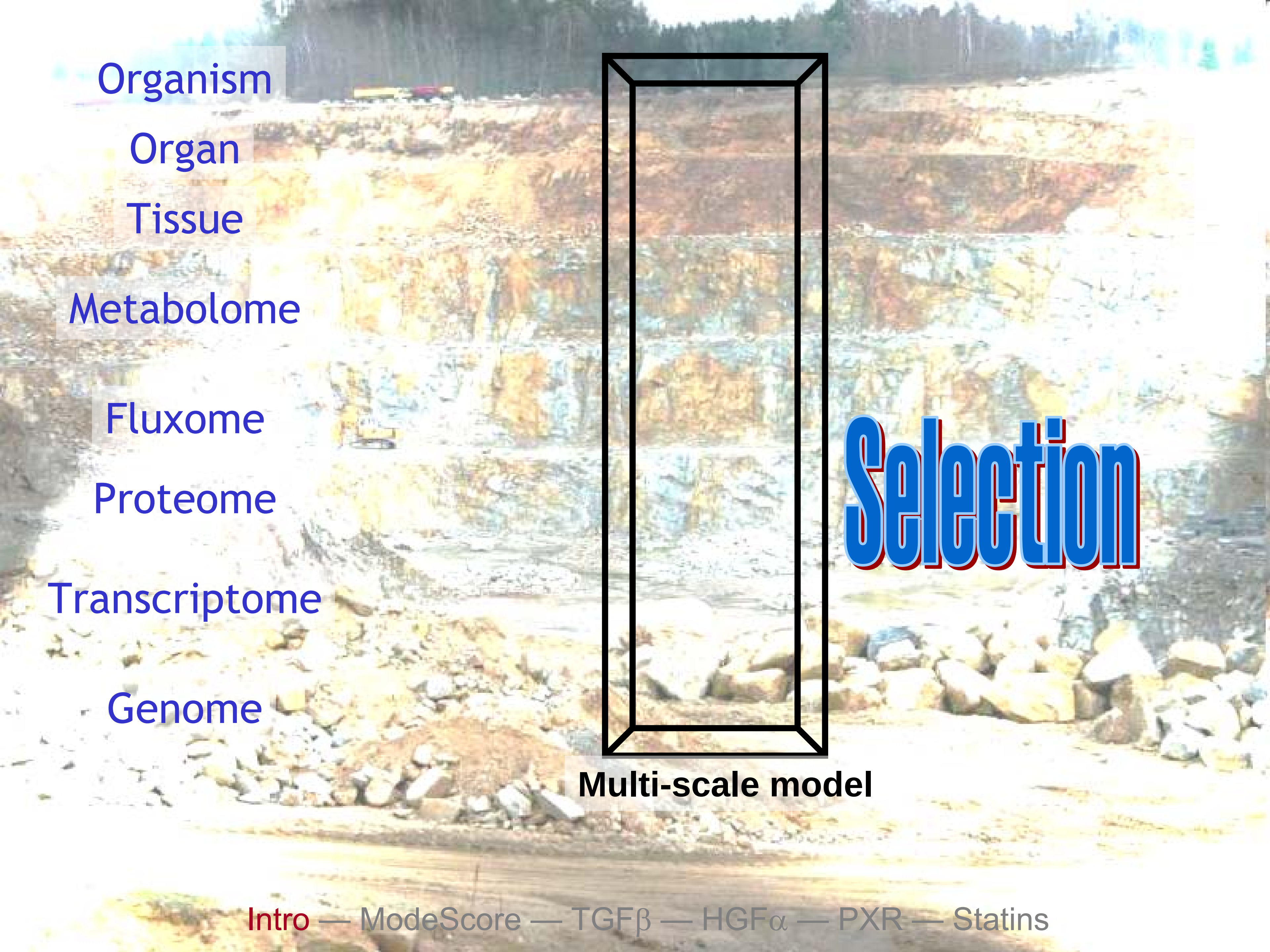


Organism

Organ

Tissue

Metabolome

Fluxome

Proteome

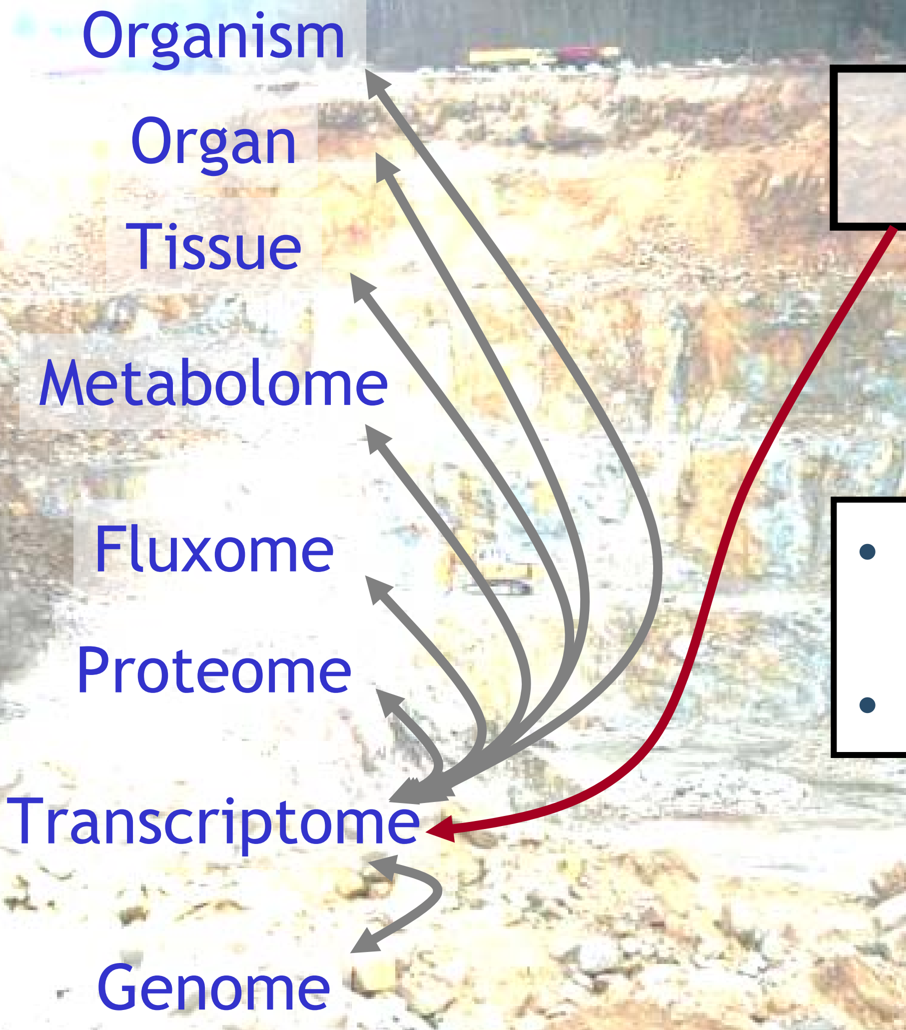
Transcriptome

Genome

Selection

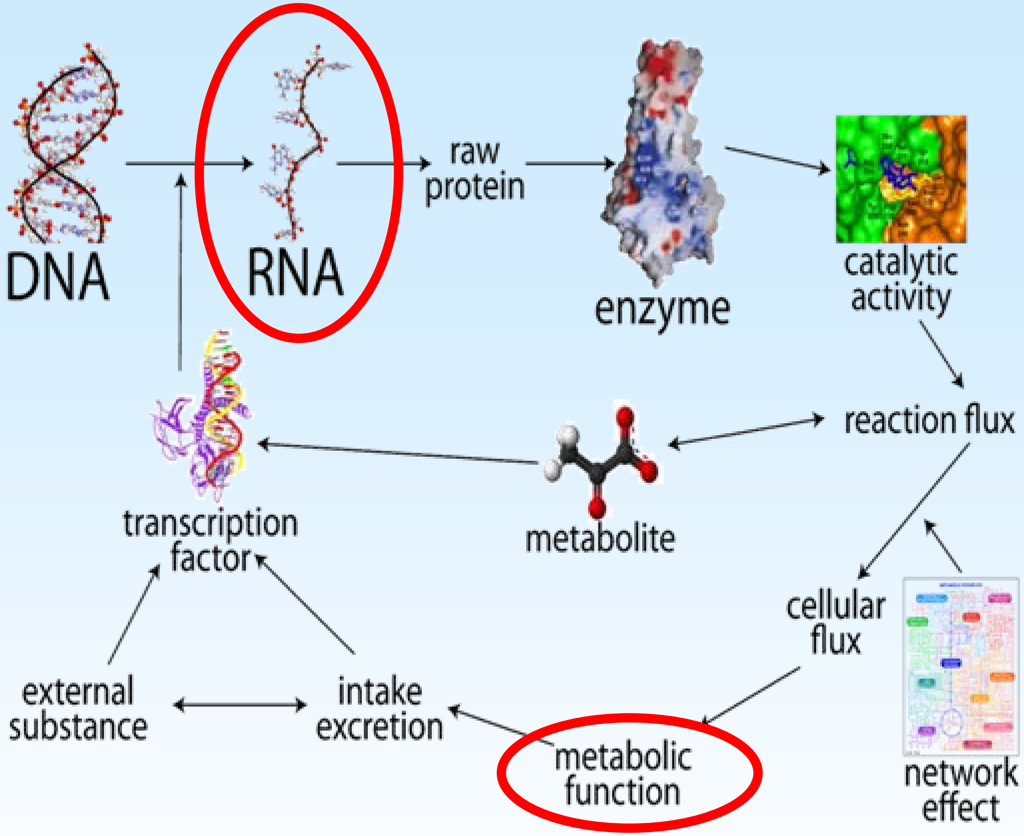
Multi-scale model

Microarrays



- large information gain per material & money
- relatively easy, flexible

Relating RNA/metabolic functions



- Many intermediate levels
- many modifying factors
- direct quantitative predictivity low

Gygi et al., 1999, Mol. Cell Biol.

additional information necessary

Hoppe, 2012, Metabolites 2(3).

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What is this?

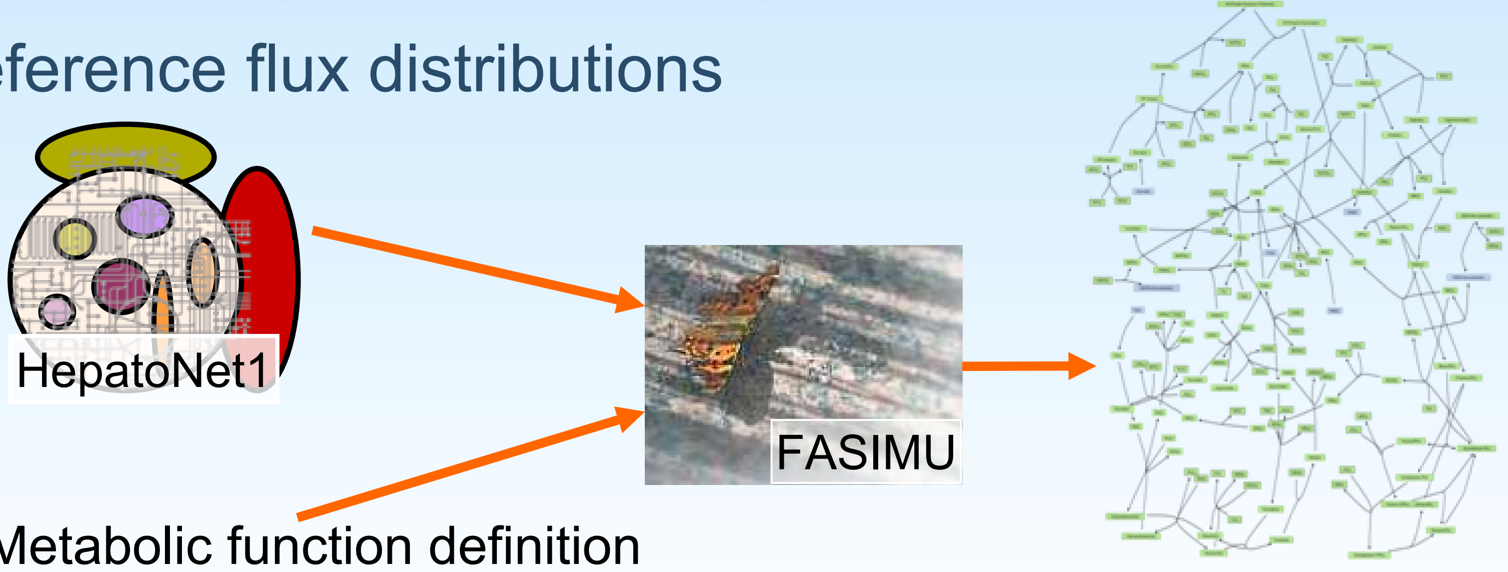
Similarity score

Prediction method

We know

- the way enzymes cooperatively work
- the cell’s metabolic functions

→ reference flux distributions



HepatoNet1

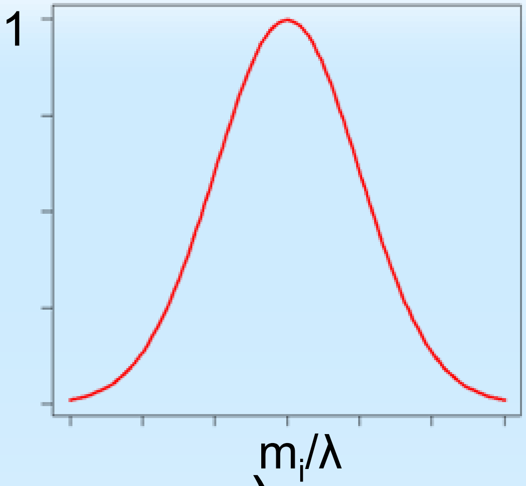
FASIMU

Metabolic function definition

Reference flux mode

HepatoNet1 … , Gille et al., 2010, Mol Syst Biol
FASIMU … Hoppe et al., 2011, BMC Bioinf

Prediction method



Assumptions:

- Gene up → flux value up (& vice versa)
- Normal distribution
- Probability maximum: flux/scaling factor λ

Pattern match

Abundance change — Flux mode

Result

Estimation of function's amplitude of change

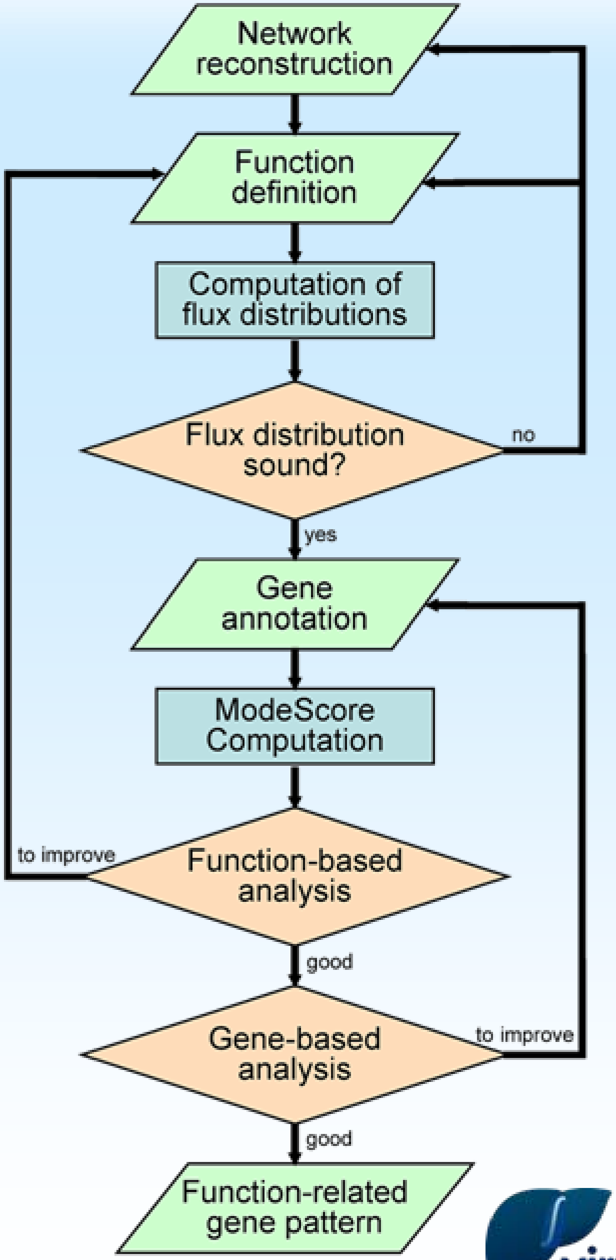
ModeScore process

Semi-automatic

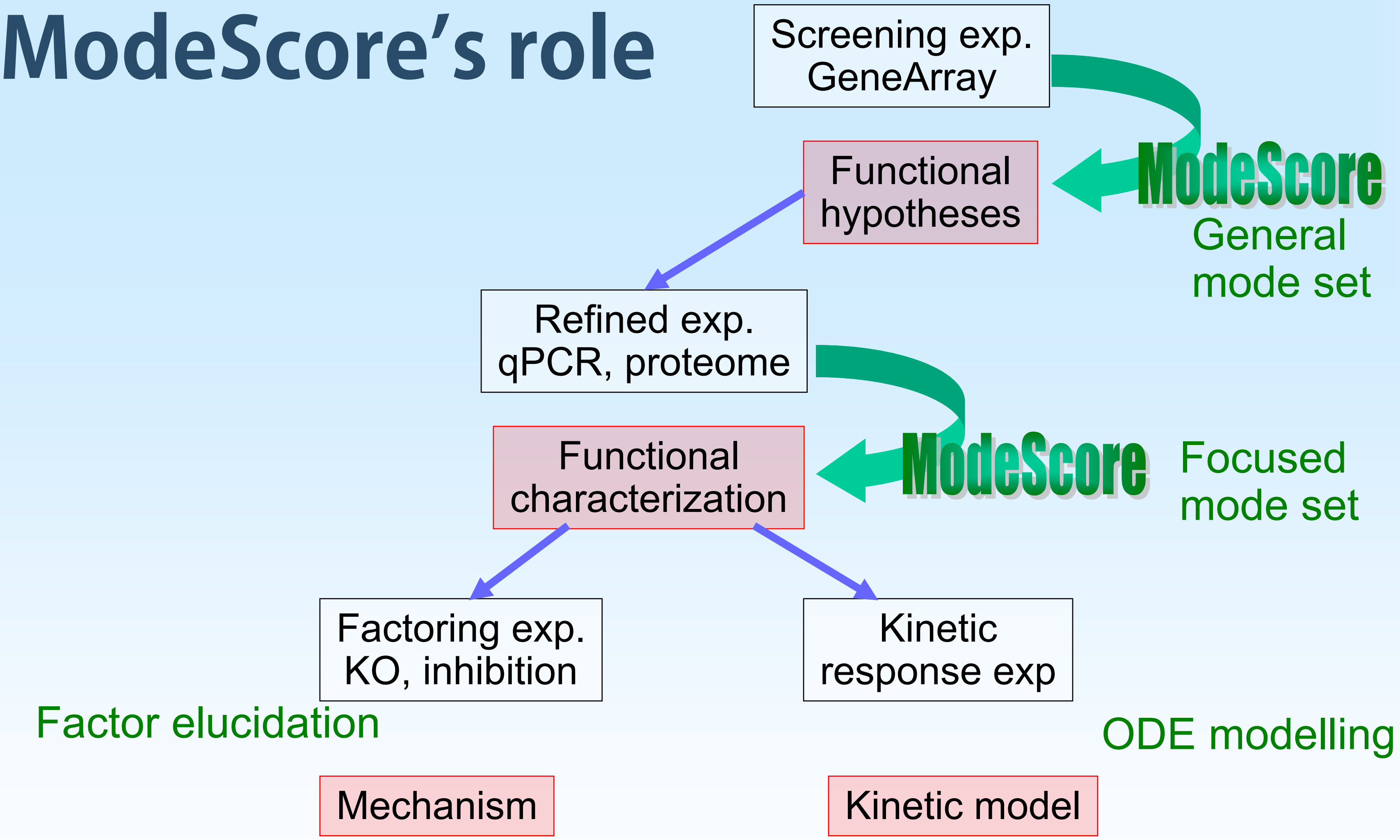
- refinement of network, functions, annotations
- scoring/ranking
- manual selection

Selection of changed genes

Testable hypothesis



ModeScore’s role



Screening exp.
GeneArray

ModeScore

General
mode set

Functional
hypotheses

Refined exp.
qPCR, proteome

ModeScore

Focused
mode set

Functional
characterization

Factoring exp.
KO, inhibition

Factor elucidation

Mechanism

Kinetic
response exp

ODE modelling

Kinetic model

virtual liver
network

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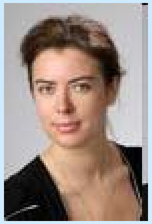
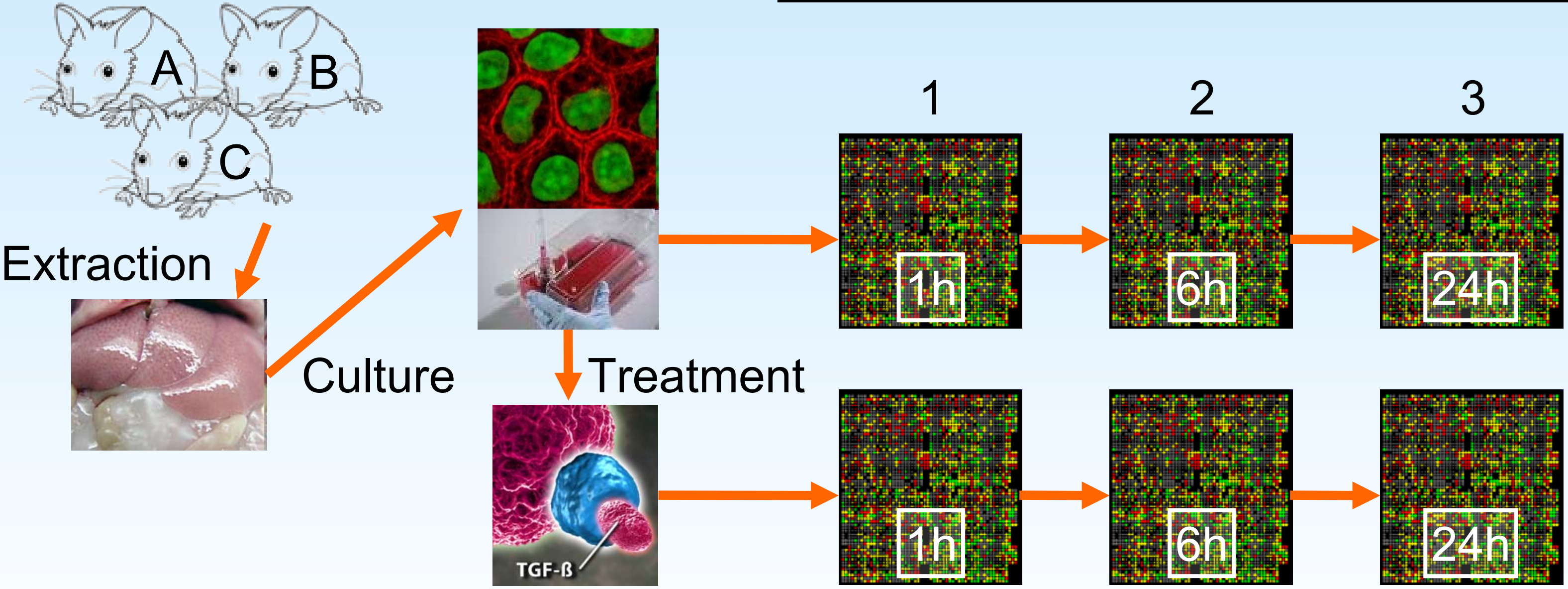
Hepatocyte culture/TGFβ treatment

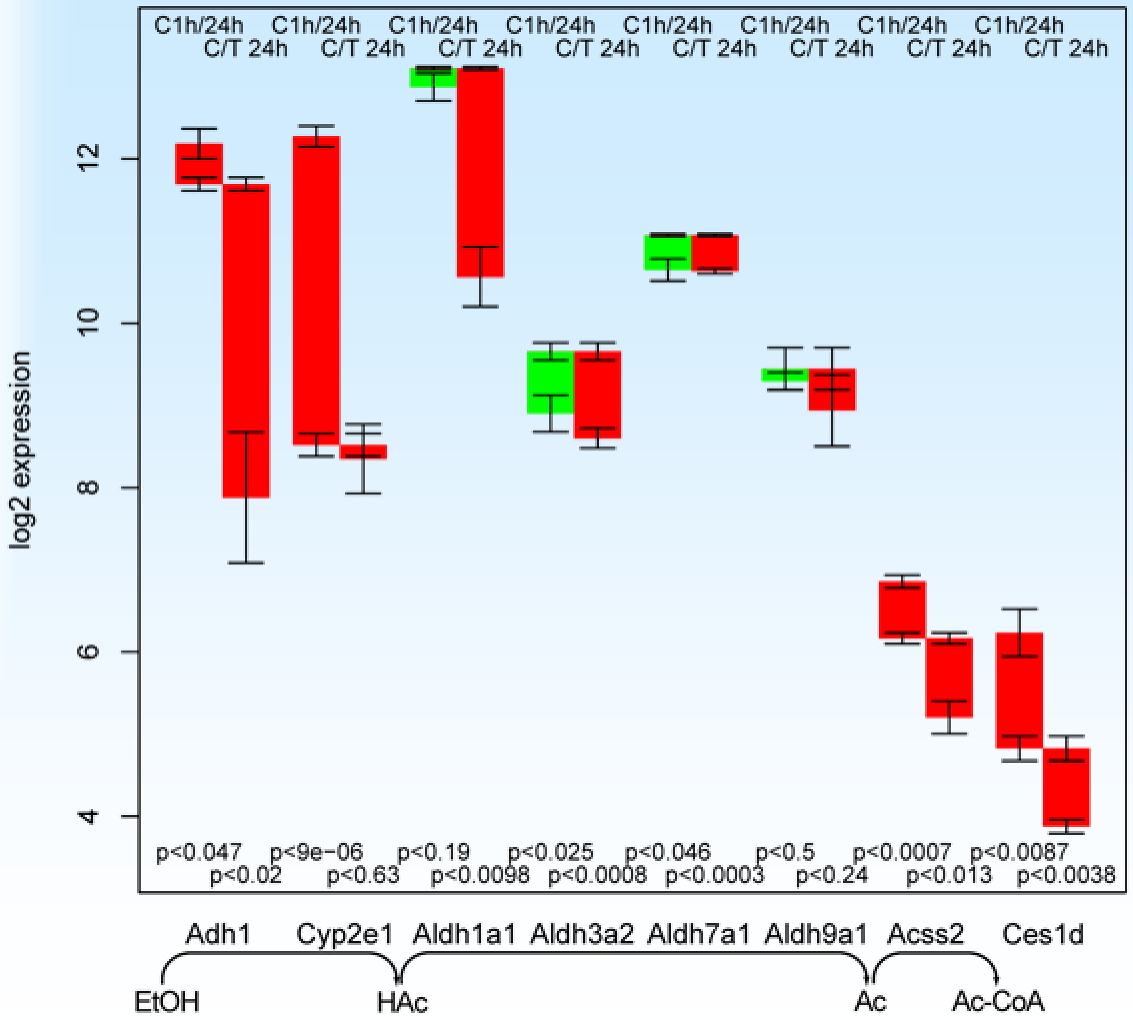


Iryna Ilkavets
Mannheim

Hoppe et al. 2012, Metabolites **2**(4).
Dooley, 2008, Gastroenterology **135**(2).



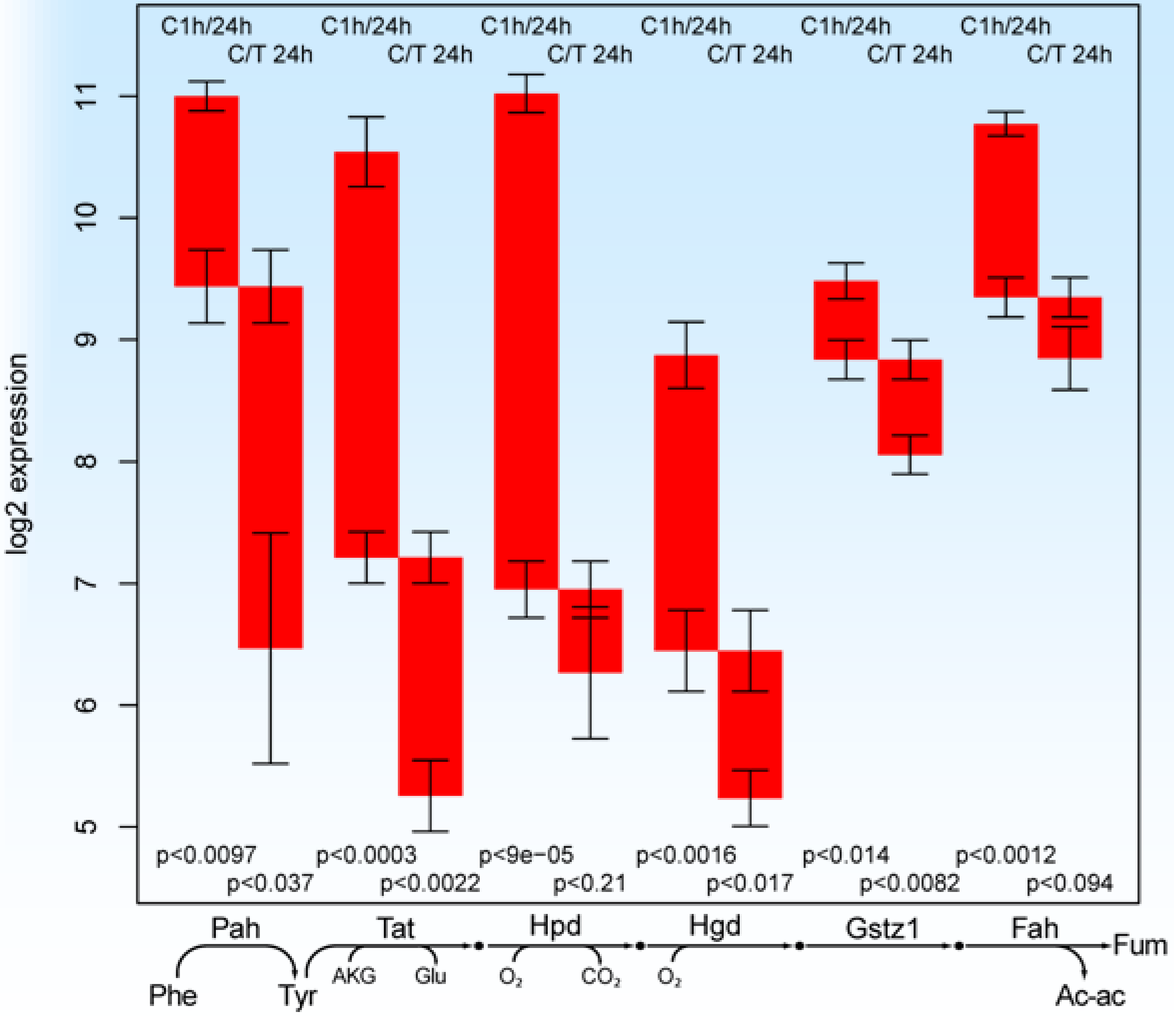
Ethanol degradation



TGFβ down-regulates main isoforms Adh1 Aldh1a1

Isoform

Phenylalanine/Tyrosine degradation



Down-regulation in time

TGFβ enhances

Very consistent

]

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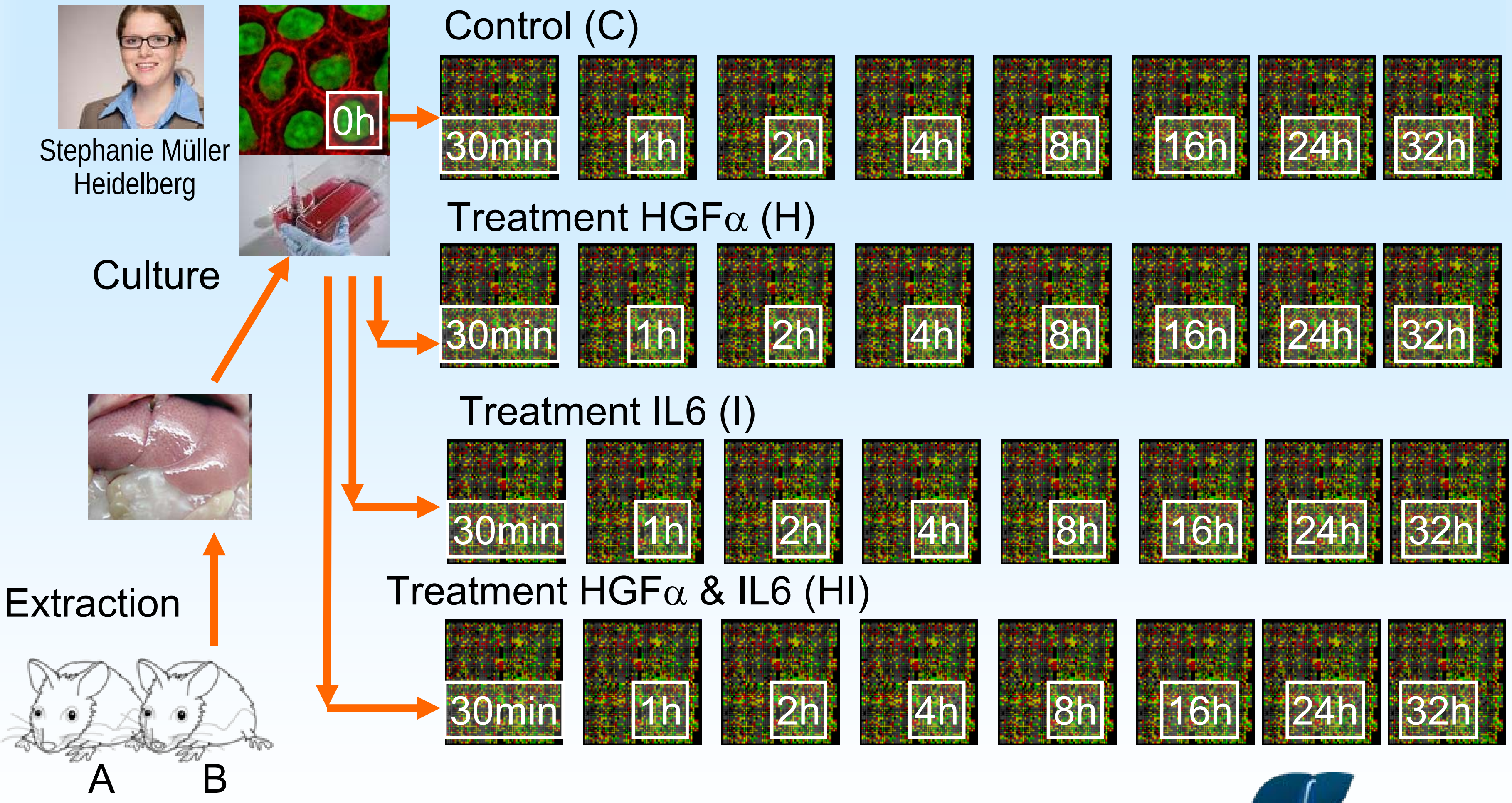


Stephanie Müller
Heidelberg

Culture

Extraction

A B

0h

Control (C)

30min 1h 2h 4h 8h 16h 24h 32h

Treatment HGFα (H)

30min 1h 2h 4h 8h 16h 24h 32h

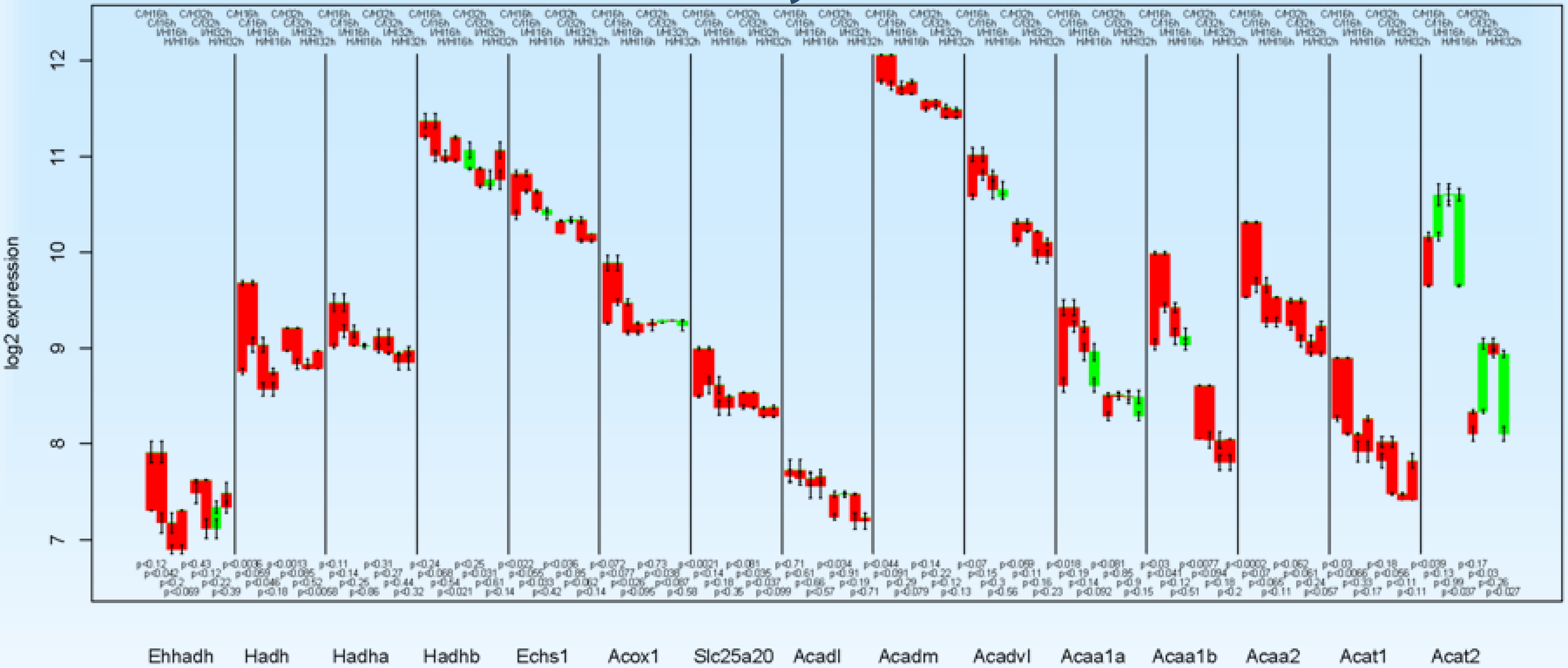
Treatment IL6 (I)

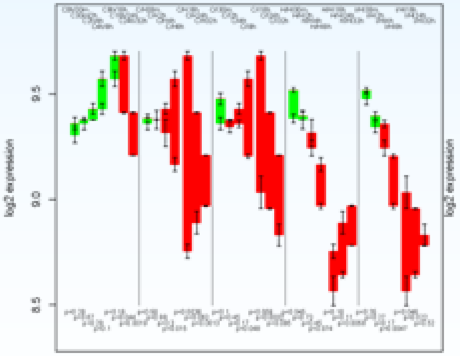
30min 1h 2h 4h 8h 16h 24h 32h

Treatment HGFα & IL6 (HI)

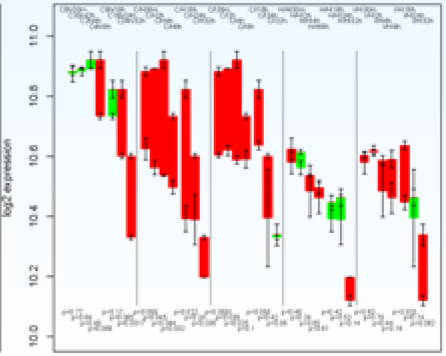
30min 1h 2h 4h 8h 16h 24h 32h

Beta oxidation down by HGFα and IL6

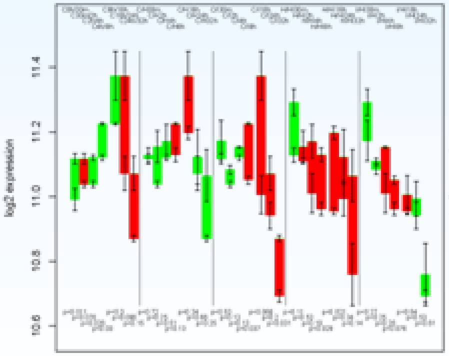




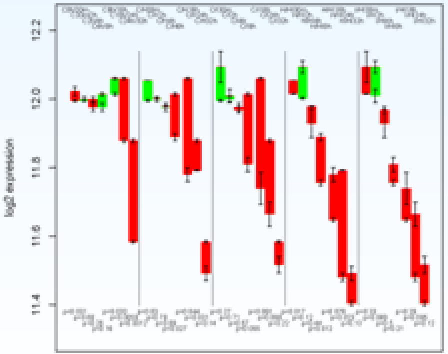
Hadh



Echs1

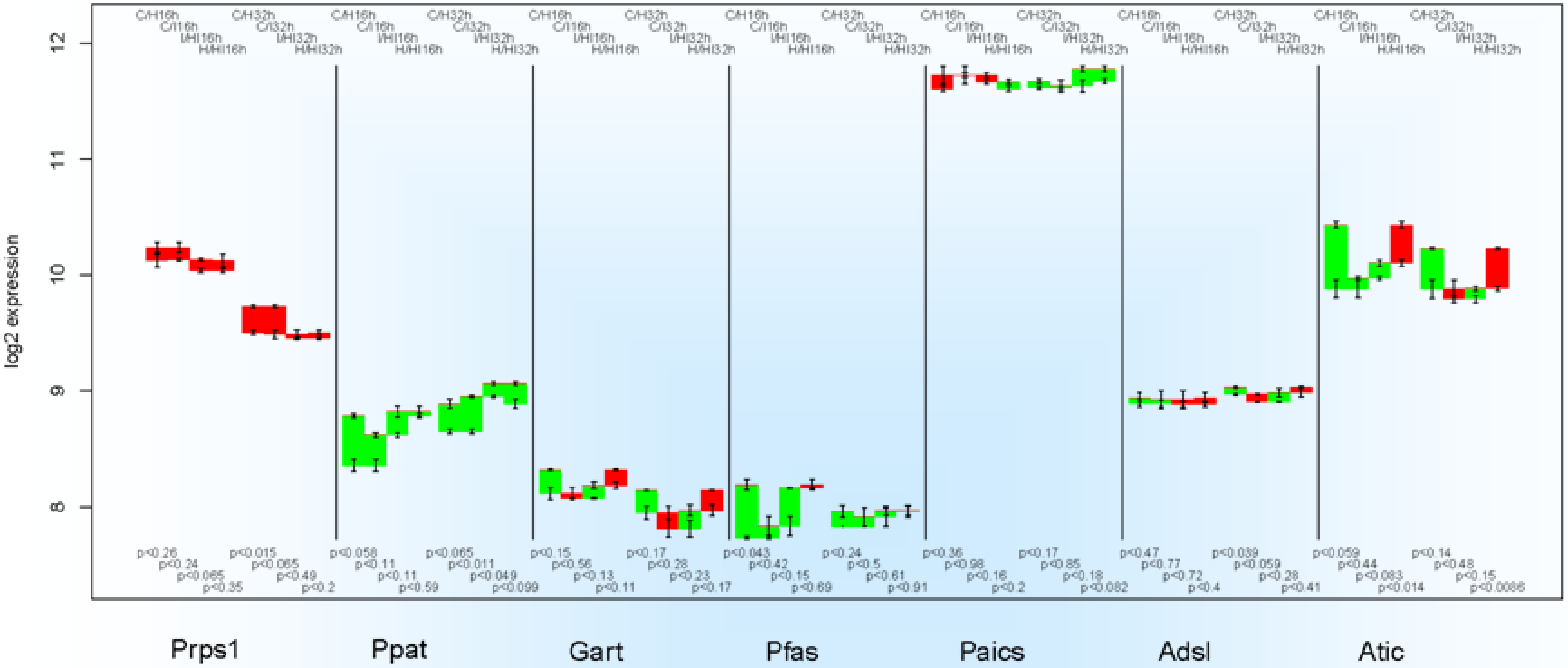


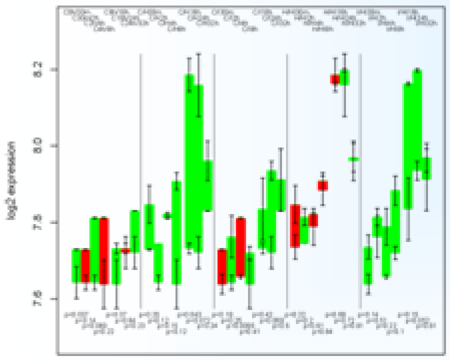
Hadhb



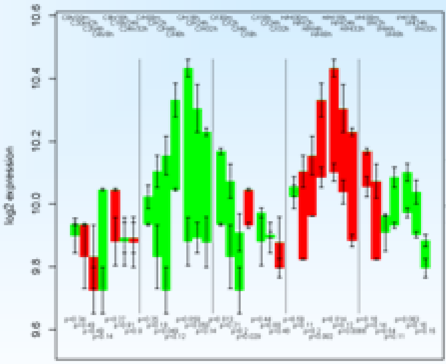
Acadm

Purine synthesis up by HGFα (less by IL6)

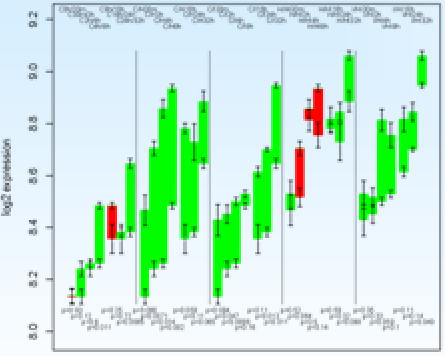




Pfas



Atic



Ppat

Gart

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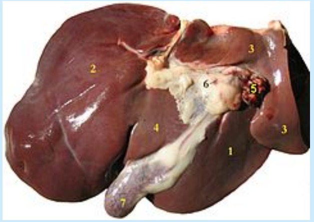
PXR/CAR/PPARα

Benjamin Kandel,
Stuttgart



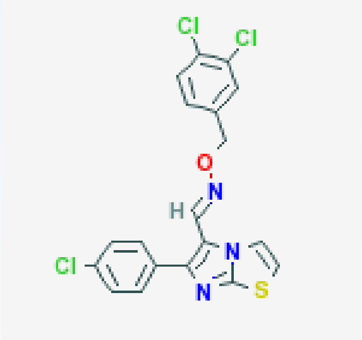
6 donors
a b c d e f

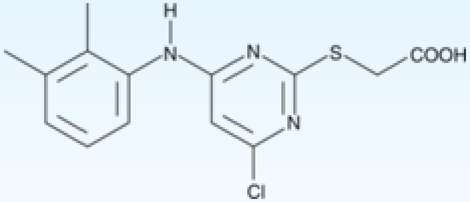
D (DMSO empty)
control

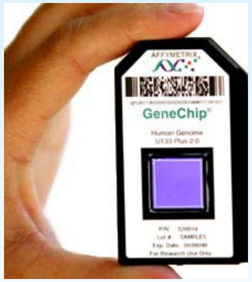
C (CITCO) ,targets
CAR (constituitive androstane
receptor)

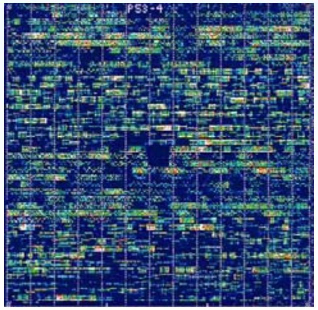
R (Rifampicin,
antibiotics)
targets ***PXR***

W (WY14643)
PPARα inducer



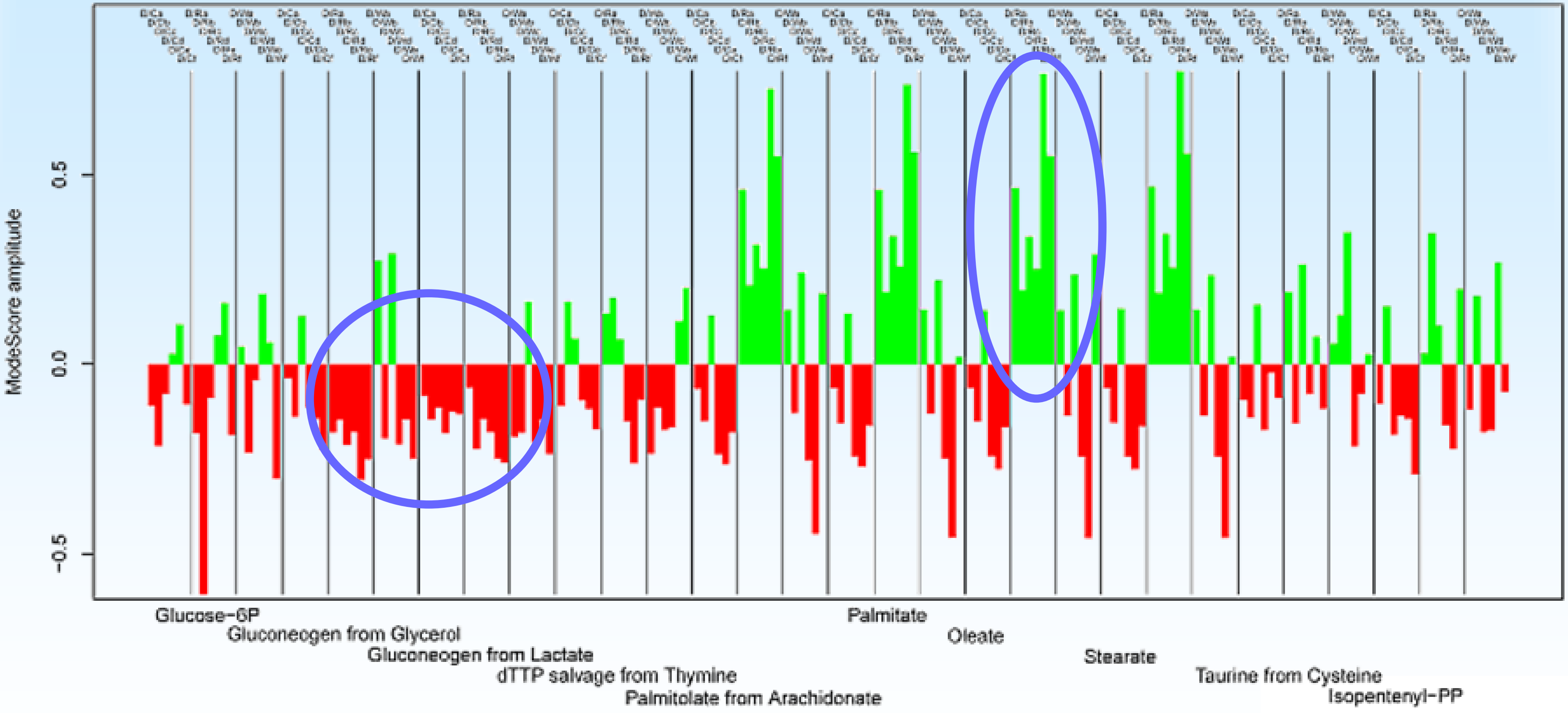




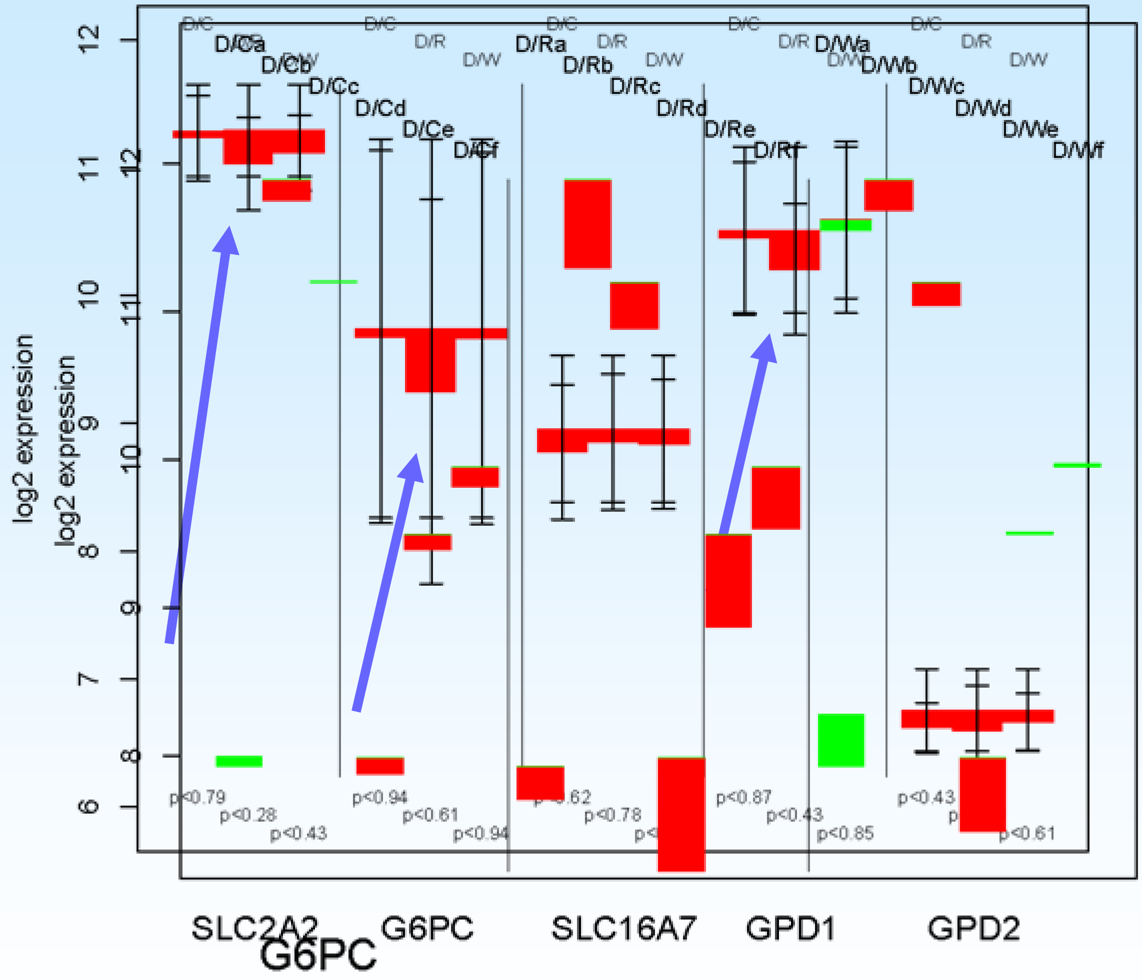


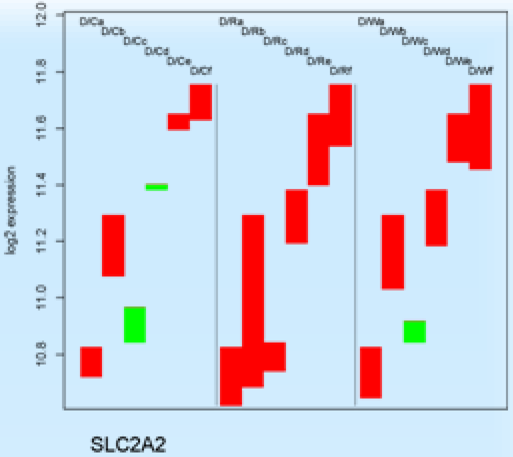
Kandel, Zanger
(Stuttgart)

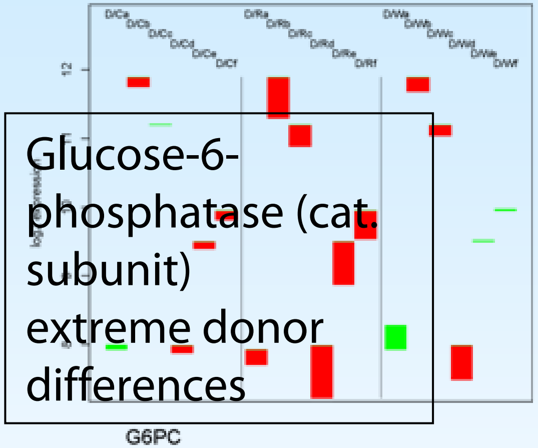
ModeScore noticeable functions



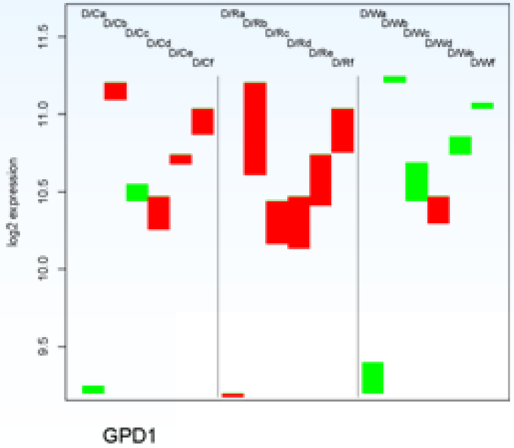
Gluconeogenesis, PXR down



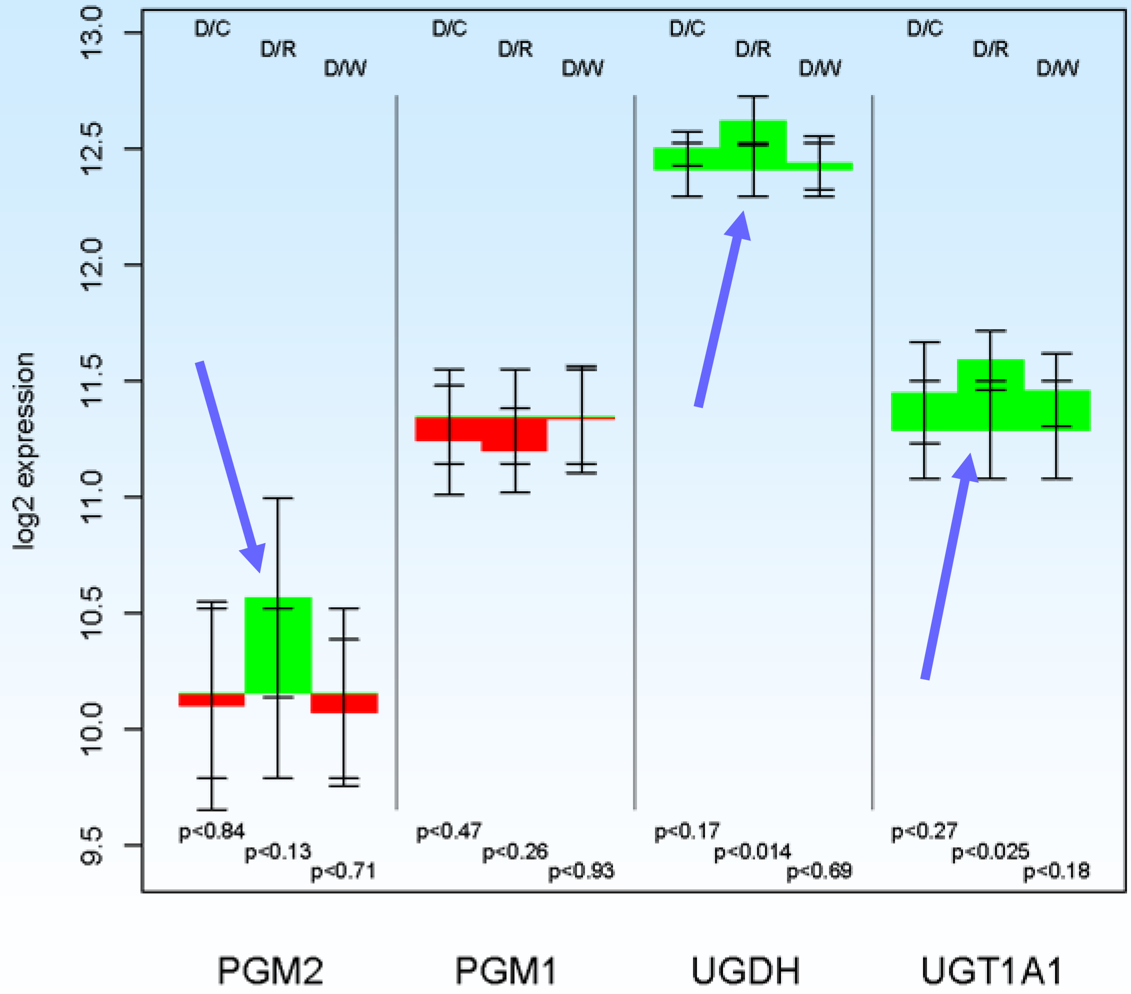


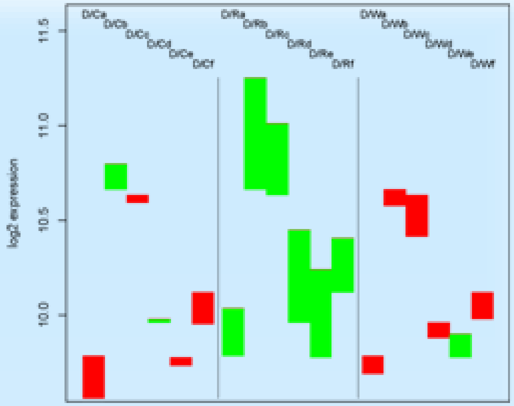


Glucose-6-phosphatase (cat. subunit) extreme donor differences

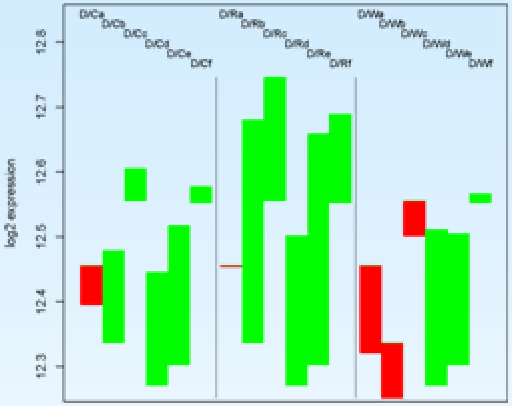


Glucuronization, PXR up

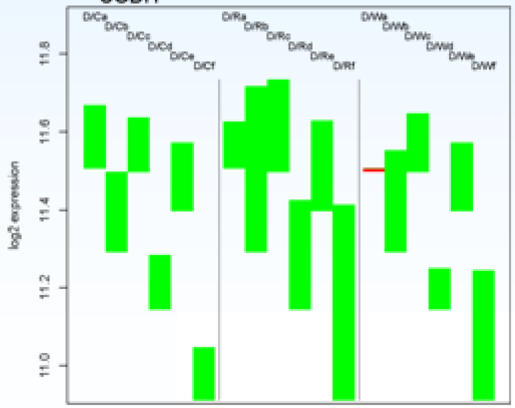




PGM2

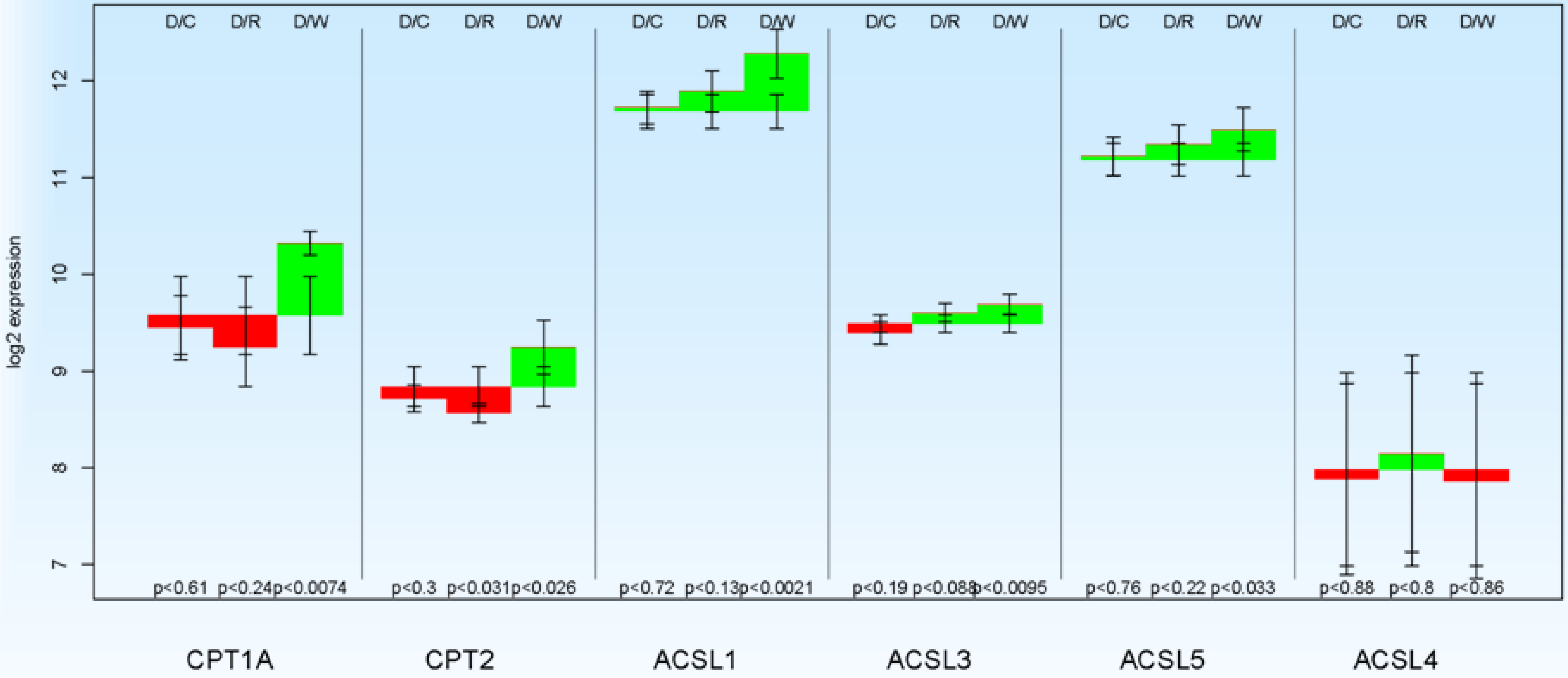


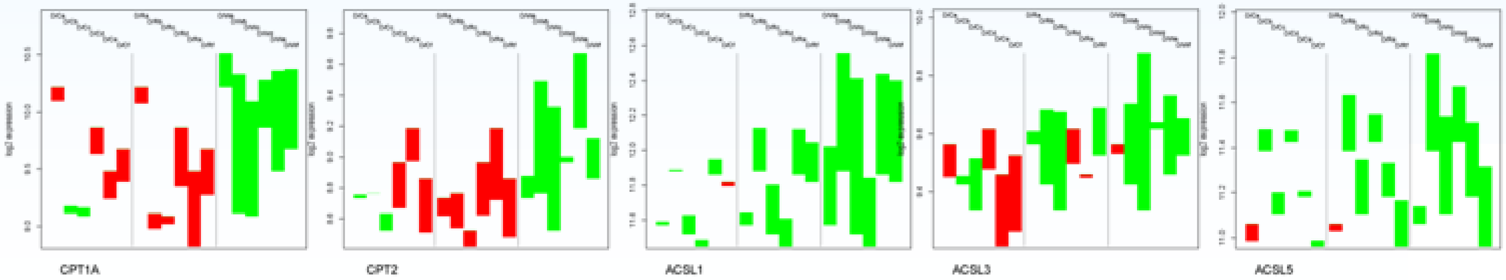
UGDH



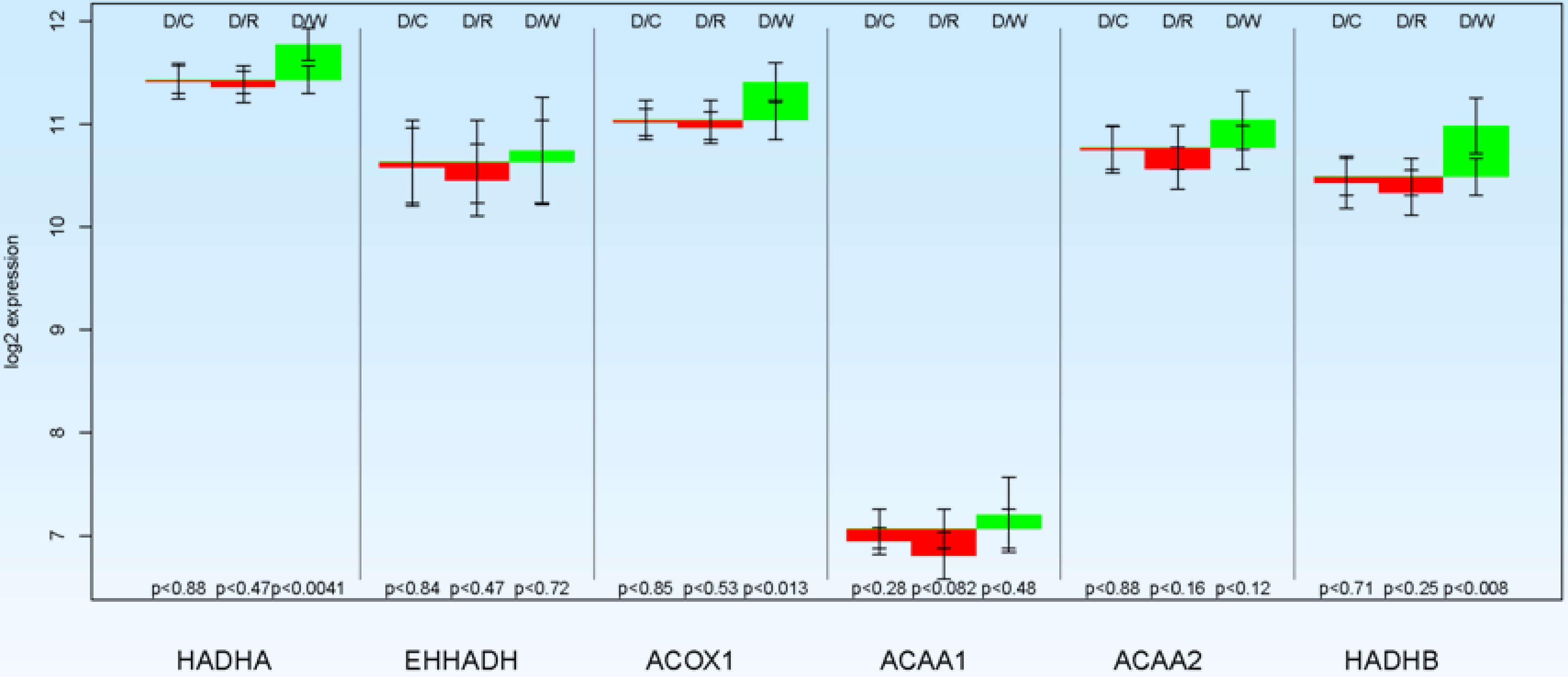
UGT1A1

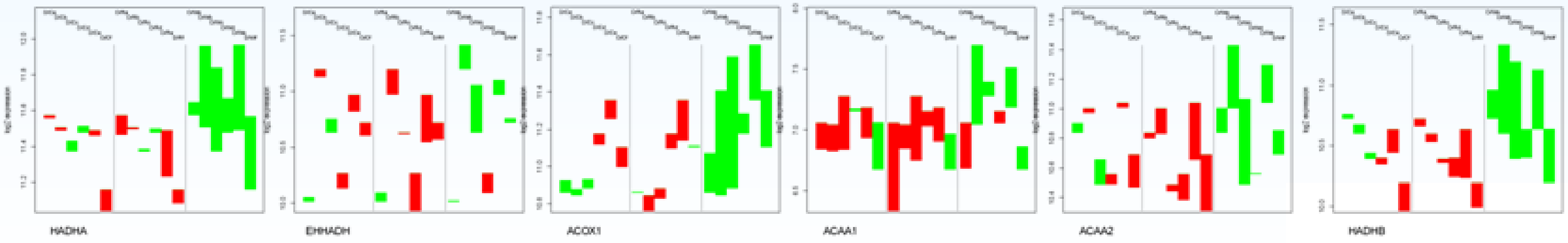
Fatty acid activation, PPARα up





Beta oxidation, PPARα up





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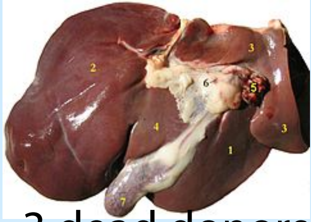
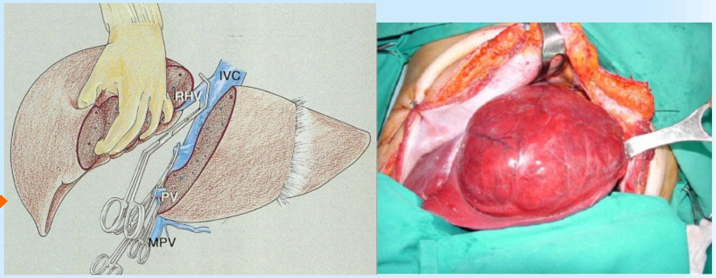
Statin exp.



3 dead donors

A B C

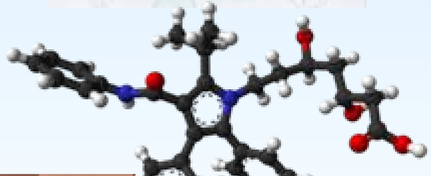


3 donors, lobectomy, liver cancer

D E F

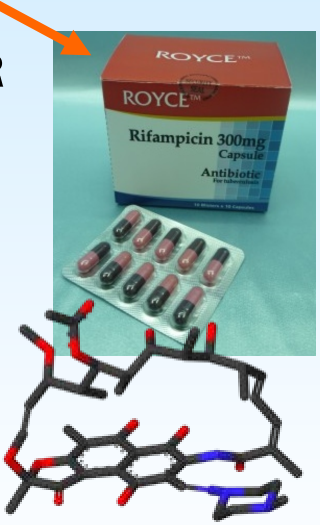
cell cultures

A

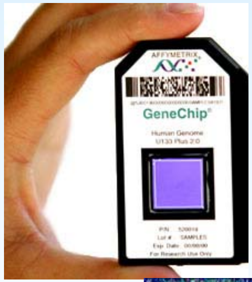


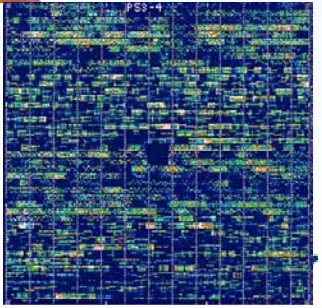
V

R



C



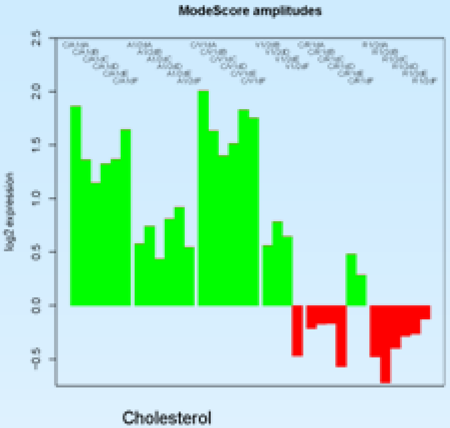


Mateja Hafner
Ljubljana

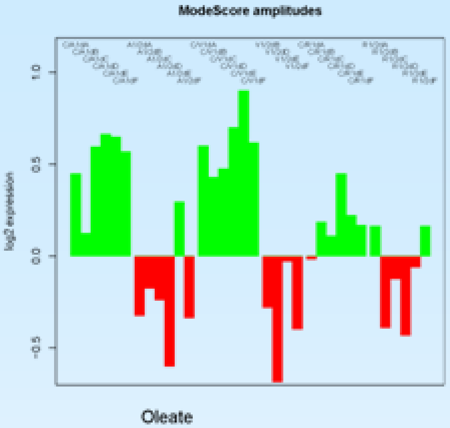
1d

2d

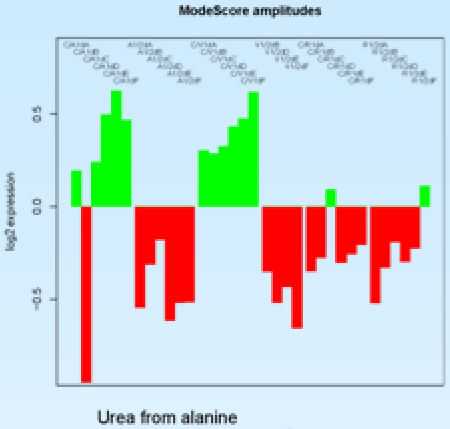
ModeScore analysis, areas of large changes



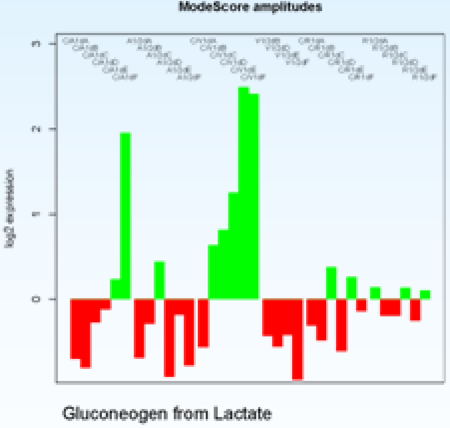
Cholesterol synthesis



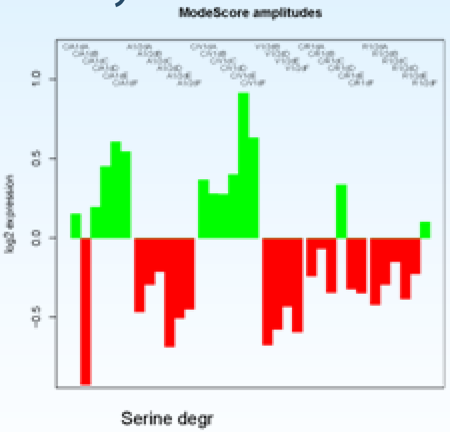
Fatty acid metabolism



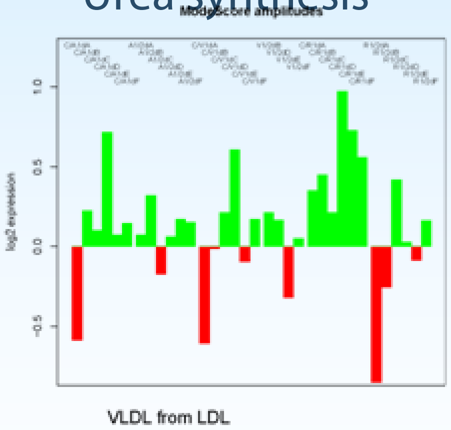
Urea synthesis



Sugar metabolism

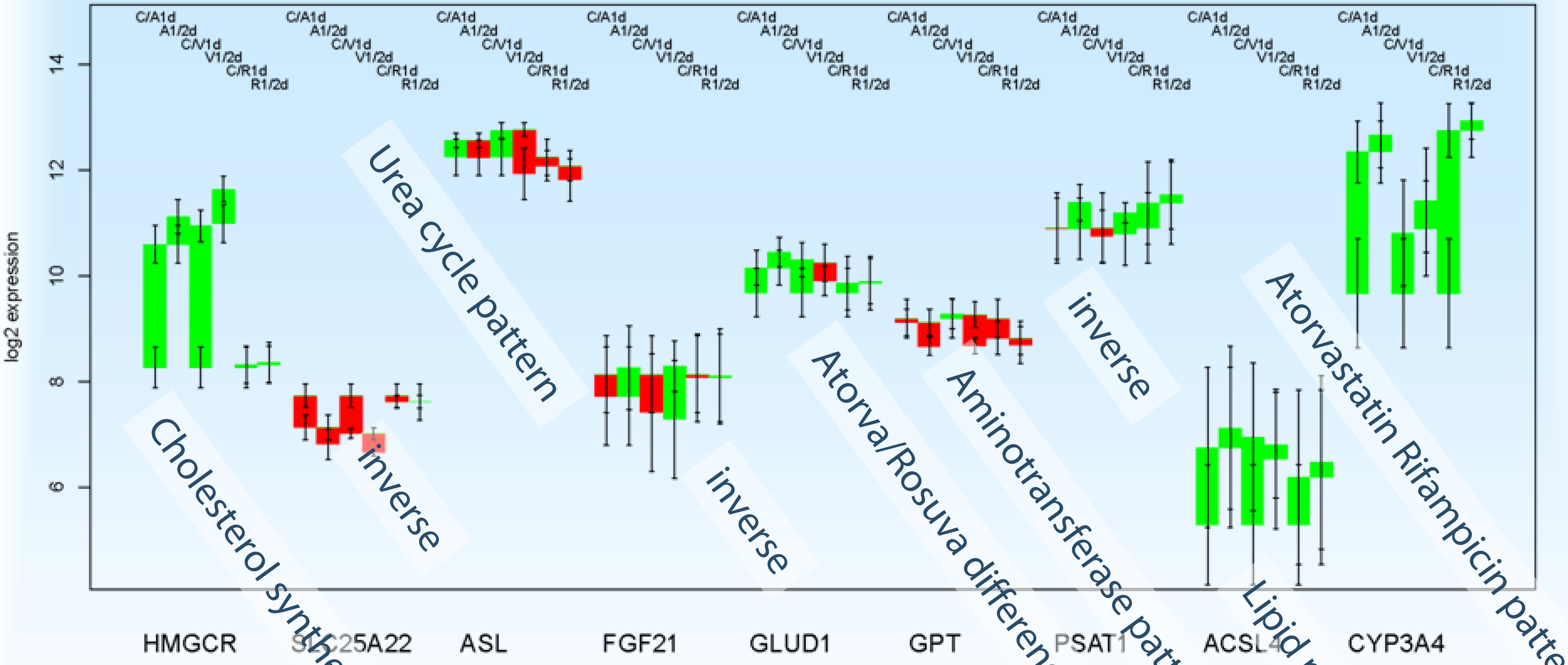


Transamination



Lipoproteins

Patterns of gene changes



Cholesterol synthesis pattern

inverse

Urea cycle pattern

inverse

Atorva/Rosuva difference pattern

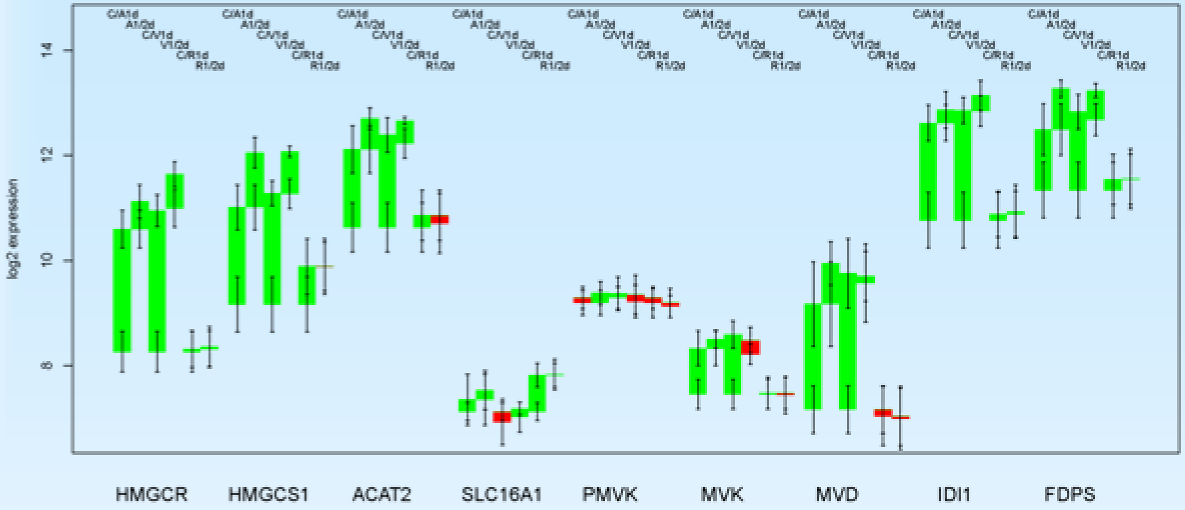
Aminotransferase pattern

inverse

Lipid pattern

Atorvastatin Rifampicin pattern

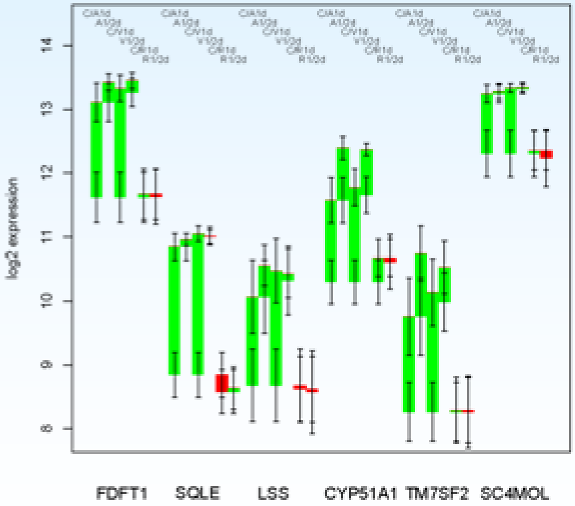
Cholesterol synthesis pattern

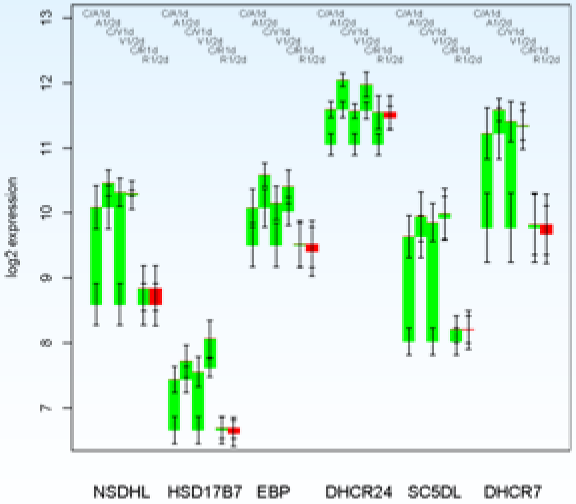


Very consistent

18/21 enyzmes of cholesterol synthesis

Large changes





Pattern definition

Statins:

1d strong up

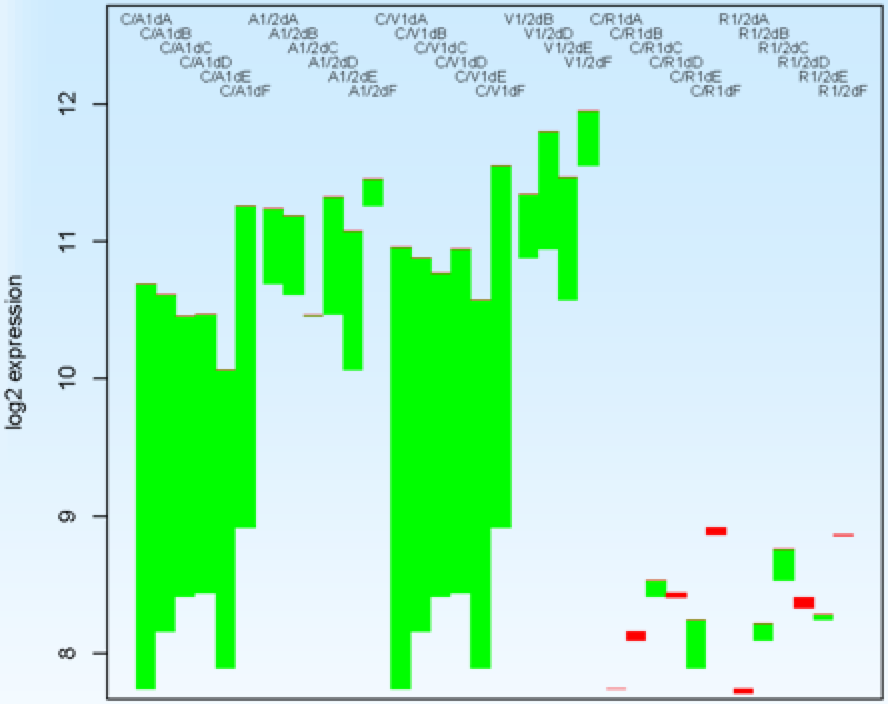
2d (slightly) up

Rifampicin:

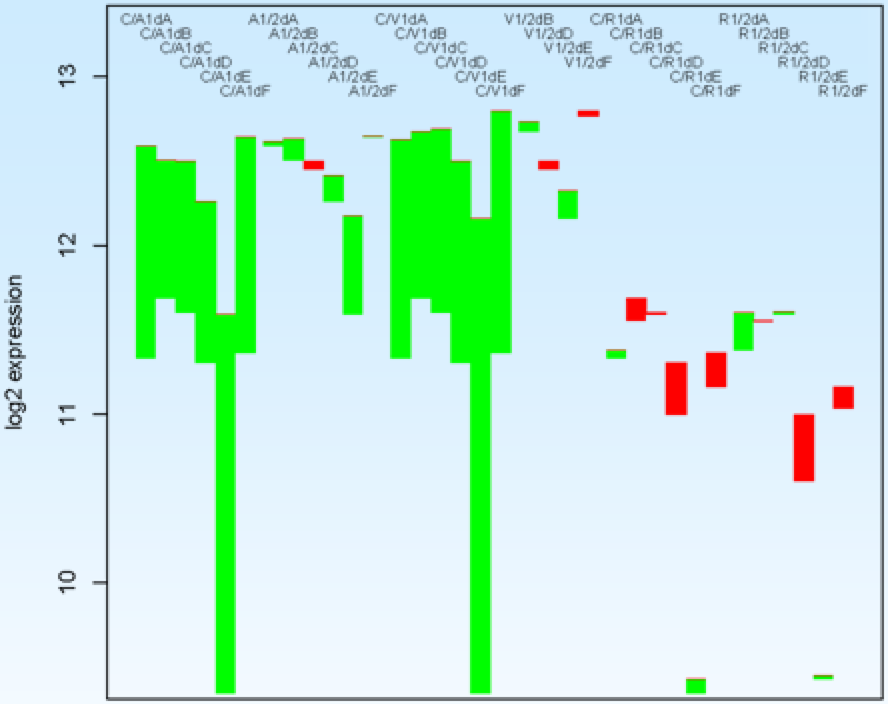
minor change

virtual liver network

Cholesterol synthesis pattern



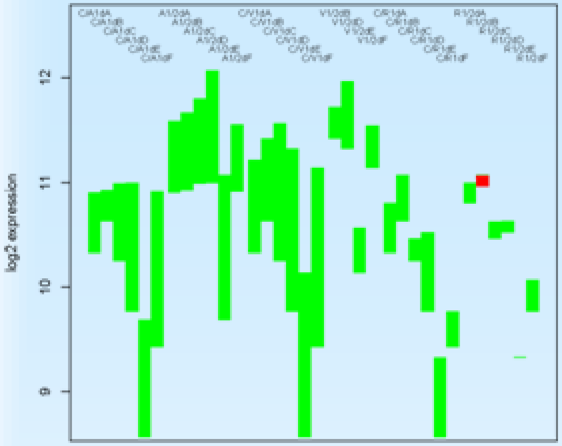
HMGCR HMG-CoA reductase



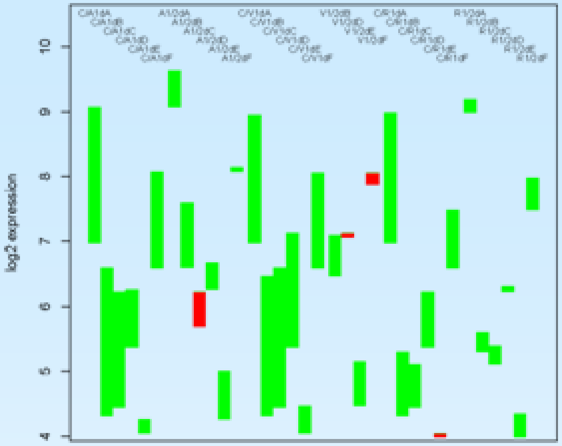
INSIG1 Insulin-induced gene

Other enzymes and genes: ACAT2, FABP1

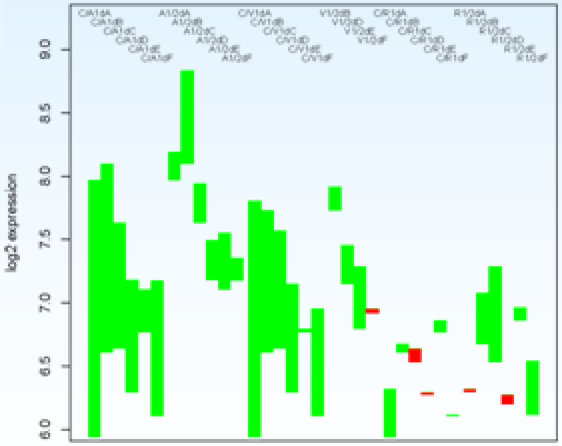
Lipid pattern



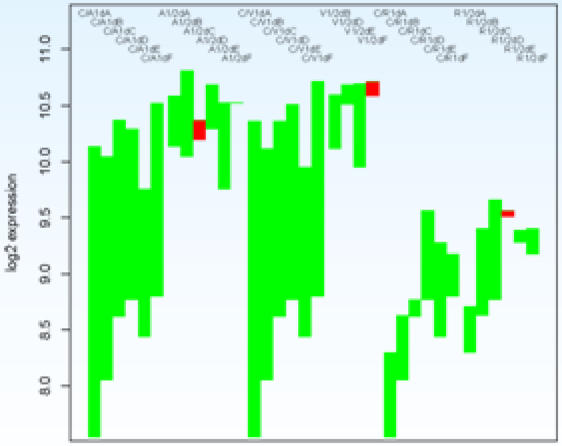
SCD δ9 desaturase



ACSL4 acyl-CoA synthetase



ELOVL2



ELOVL6

FA elongases

Particular enzymes in lipid formation, FA synthesis and degradation

Specific isoforms

Pattern definition

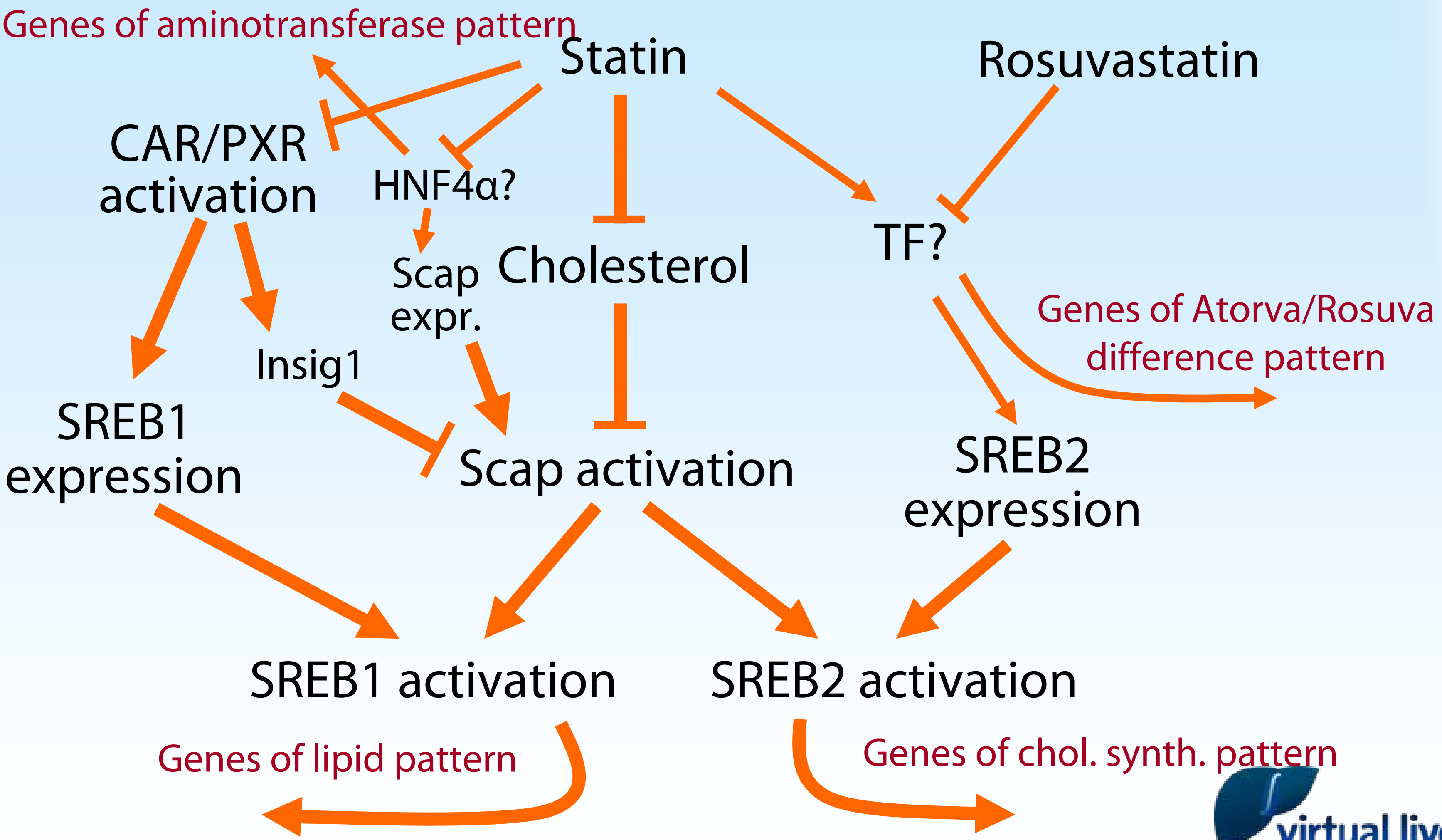
Statins:

- 1d up
- 2d up

Rifampicin:

- up, less than statins

Link of sterol and lipid regulation



Genes of aminotransferase pattern

Statin

Rosuvastatin

CAR/PXR activation

HNF4α?

Scap expr.

Cholesterol

TF?

Genes of Atorva/Rosuva difference pattern

Insig1

SREB1 expression

Scap activation

SREB2 expression

SREB1 activation

SREB2 activation

Genes of lipid pattern

Genes of chol. synth. pattern

virtual liver network

Take-home messages

- Gene arrays suitable as hypothesis-finding source
- ModeScore: hypotheses on metabolic functions
- Hypotheses just based on tramscripts:
  - TGFβ, tyrosine degradation down
  - HGFα, IL6, beta oxidation down
  - PPARα, beta oxidation up
  - PXR, glucuronization capacity up
  - Statins, wide-range regulation effects

mice

human

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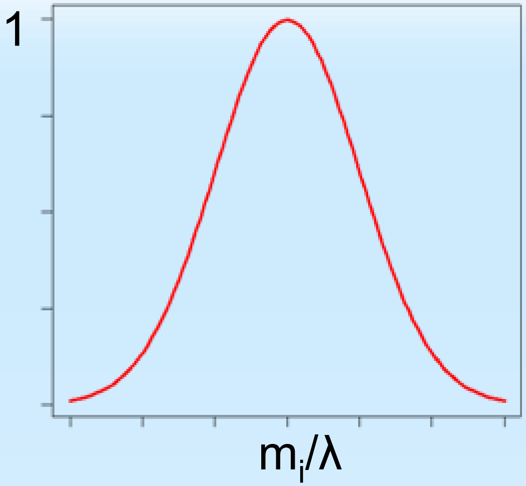
www.charite.de/sysbio/people/hoppe
www.bioinformatics.org/fasimu
ModeScore method: Hoppe & Holzhütter, 2012, OASIcs, **26**.
Review on transcript data use: Hoppe, 2012, Metabolites **2**(4).

Mode set scoring



$\mathrm{Score}(M_k, V) = \frac{\sum_{i \in I_k} w_i \mathrm{score}_i(m_i, v_i)}{\sum_{i \in I_k} w_i}$	total score
$I_k = \{i \mid m_i \neq 0\}$	indices of nonzero values
$w_i = \sqrt{\lvert m_i \rvert \omega_i}$	weights
ω_i	weight adjustment
$\mathrm{score}_i(m_i, v_i) = e^{-\frac{1}{2}\left(2\frac{\lambda v_i - m_i}{\lvert m_i \rvert}\right)^2}$	score component
$M_k = (m_i)_i$	k-th reference mode
$V = (v_i)_i$	relative expression profile
λ chosen such that	$\mathrm{Score}(M_k, V)$ is maximal

ModeScore amplitude $(1/\lambda)$

- Measures strength of regulation for the function
- Compatible to $\log_2$ fold change
- Cluster point (not average) of gene changes

Contribution scores $(score_i)$

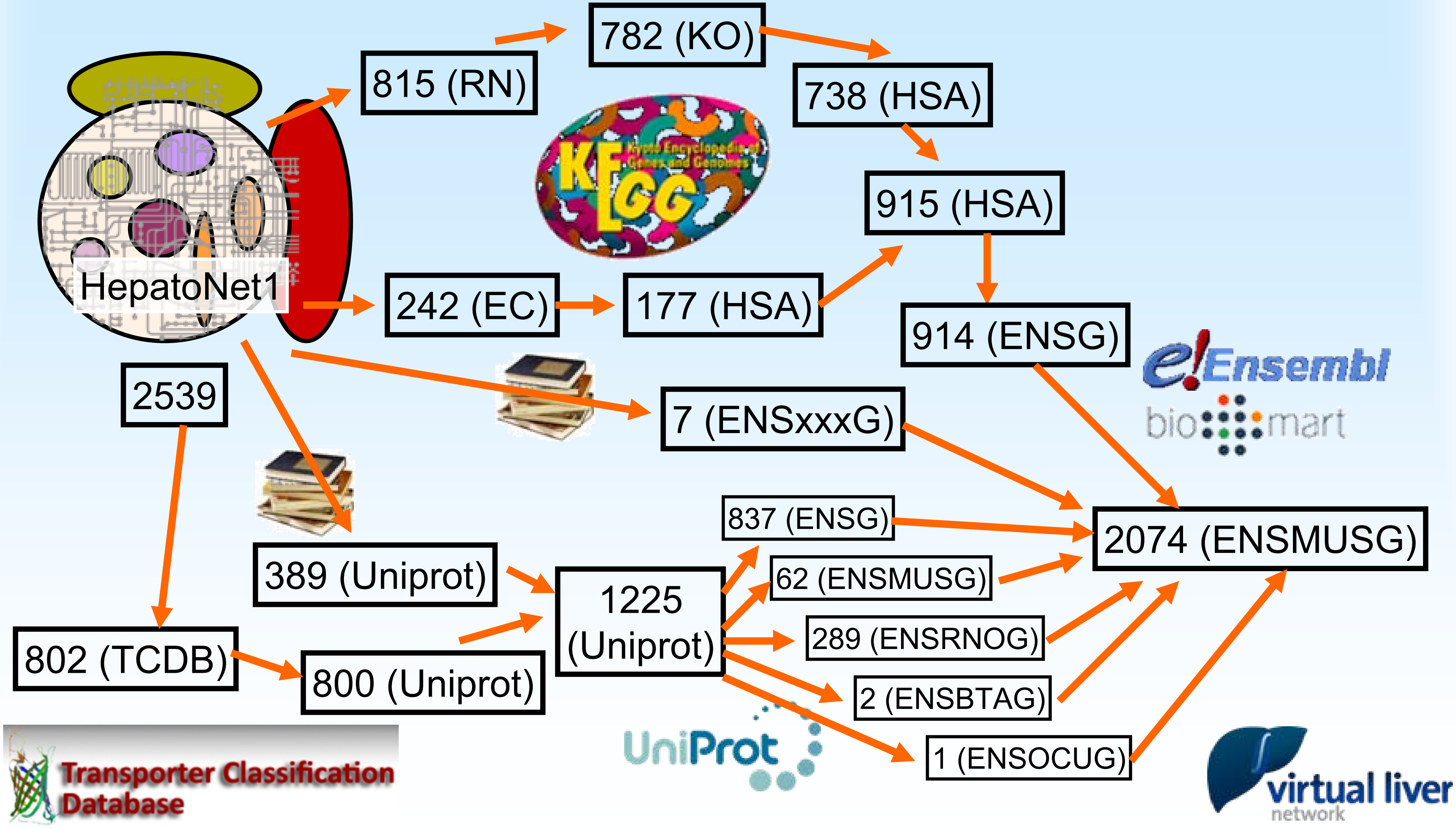
- Measures how good a gene change represents the function's amplitude

Hoppe & Holzhütter, 2012, OASIcs, **26**.

Implementation

- Reference flux mode computation
  - www.bioinformatics.org/fasimu
- ModeScore computation
  - data handling: bash/gawk
  - scaling factor optimization: octave
  - table generation: LaTeX
  - bargraphs, t-test: R

HepatoNet gene annotation



HepatoNet1

815 (RN)

782 (KO)

738 (HSA)

915 (HSA)

242 (EC)

177 (HSA)

914 (ENSG)

2539

7 (ENSxxxG)

389 (Uniprot)

802 (TCDB)

800 (Uniprot)

1225 (Uniprot)

837 (ENSG)

62 (ENSMUSG)

289 (ENSRNOG)

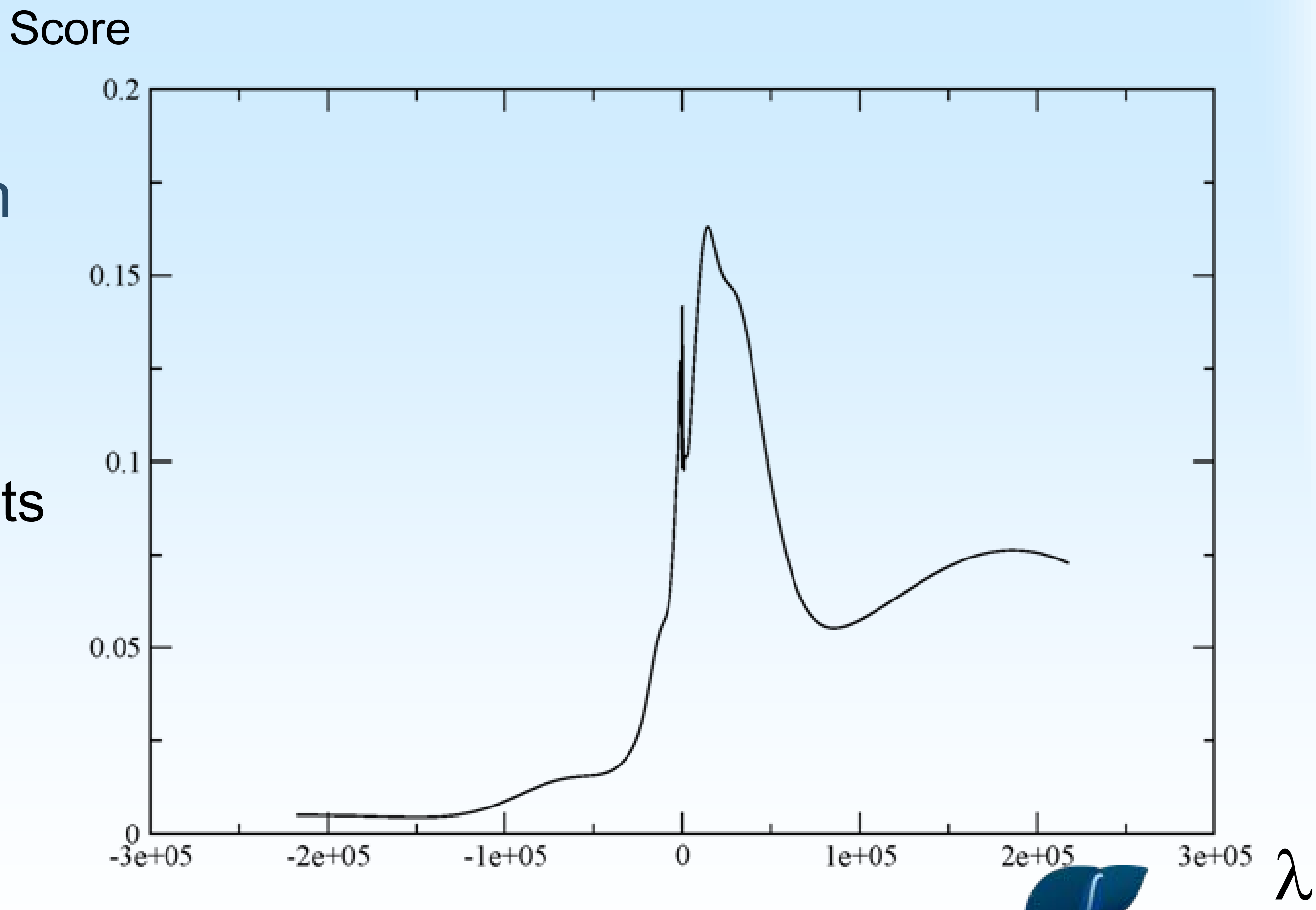
2 (ENSBTAG)

1 (ENSOCUG)

2074 (ENSMUSG)

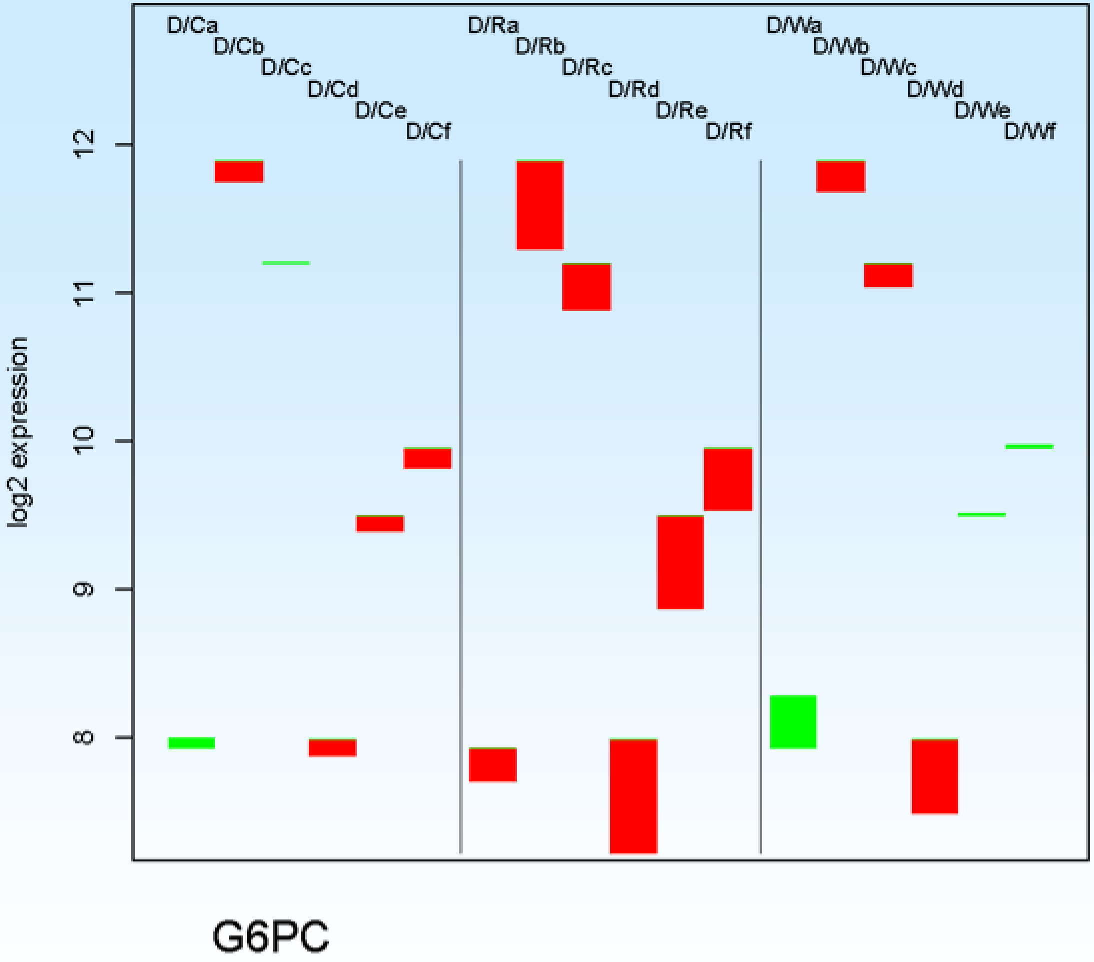
Optimization of scaling factor

- Optimization
  - Explicit derivation
  - Maxima of components
- Local maxima: alternative matches



virtual liver network

Glucose-6-phosphatase (cat. subunit)



Glucose-6-phosphatase (cat. subunit)
extreme donor differences