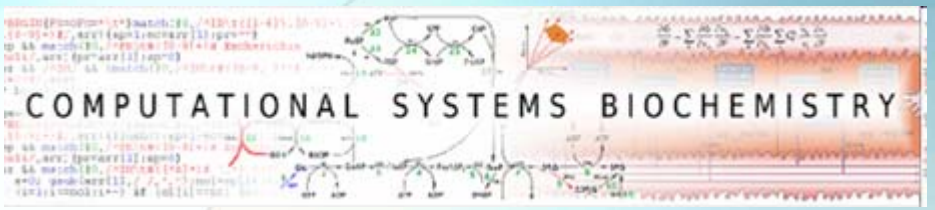

CHARITÉ
UNIVERSITÄTSMEDIZIN BERLIN

HepatoNet

Stoichiometric model of the human hepatocyte

Curation and applications

Andreas Hoppe, Charité Universtitätsmedizin Berlin
Computational systems biochemistry group

Contents

- Introduction
- HepatoNet curation
- HepatoNet applications
  - O_2 demand of NH_3 detoxification
  - Zonation
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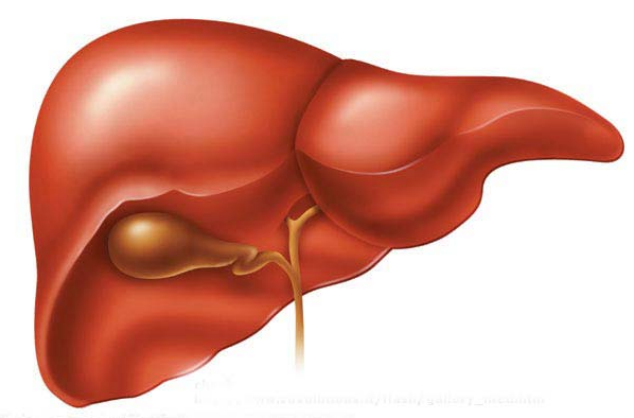
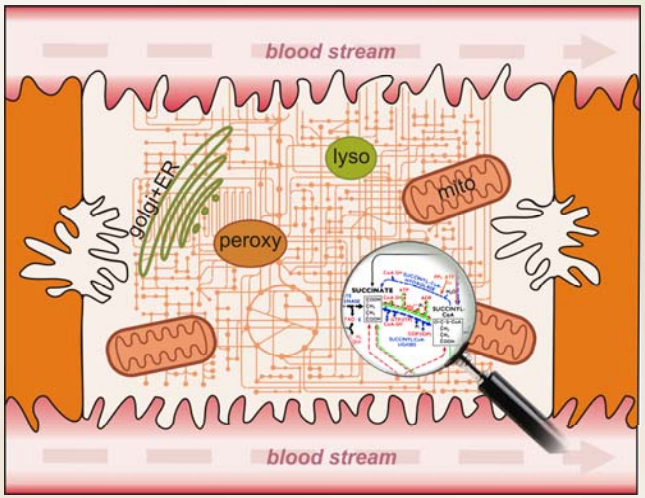
Systems Biology

- Critical voices have been raised
- Admitted: yield by systems biology is low (marketed drugs, crops etc.)
- But: contribution to better understanding hard to measure
- Investment in the future

Aims

- Biomedical modeling of liver-related diseases
- Metabolic network of the human hepatocyte as a important resource
- Part of HepatoSys
- Applications projected in VirtualLiver





Genomic approach

- High throughput (HT)
  - genetics & transcriptomics
- Reconstruction of human metabolism
  - Genome-based
  - 2,766 metabolites
  - 3,311 reactions
  - “Omnipotent” cell

Duarte et. al. Global reconstruction of the human metabolic network based on genomic and bibliomic data PNAS 2007; 104(6): 1777ff.

Transcription-based network

- Expression data
  - Primary hepatocytes
- Prediction
  - Flux-balance condition
  - Maximal match
- Tissue specific network

Shlomi et al. Network-based prediction of human tissue-specific metabolism.Nat Biotechnol. 2008; 26(9):1003ff.

Metabolic functions (123)

Purines and pyrimidines
- rephosphorylation
- de-novo synthesis
- salvage
- regeneration of NAD(P)H redox potential

Sugar metabolism
- gluconeogenese
- glycogenesis
- glycogenolysis
- sugar degradation
- formation of nucleotide-activated sugars
- formation of aminosugars
- formation of other sugars

Amino acids – proteins - nitrogen
- formation of non-essential amino acids
- complete degradation of amino acids
- plasmaprotein biosynthesis
- ureogenesis
- creatine biosynthesis

Lipids - Sterol
- phospholipid biosynthesis
- sphingolipid biosynthesis
- fatty acid biosynthesis
- triglyceride biosynthesis
- cholesterol biosynthesis
- farnesylpyrophosphate biosynthesis
- ketogenesis
- VLDL formation
- LDL catabolism
- Bile formation

Miscellaneous
- heme biosynthesis
- biosynthesis of other cofactors

Detoxification
- formation of glutathione
- detoxification of reactive oxygen species
- bilirubin catabolism
- detoxification of xenobiotics

Metabolic functions (examples)

- Aerobic phosphorylation of ATP
  - Objective: cytosolic ATP
  - Input: O_2, Glucose, cytosolic ADP and P_i
  - Output: CO_2
- VLDL lipoprotein
  - Objective: VLDL formation
  - Input: essential substrates
  - Output: typical excretion products

Surprise: not a single function is satisfied by the genomic approach!

- Why?
  - Unsufficient transcriptomics data
  - Incomplete assignments of gene-reaction
  - Threshold issue
  - Ambiguity
    - Paralogous genes (isoenzymes)
    - Multispecific proteins
- Manual curation necessary!

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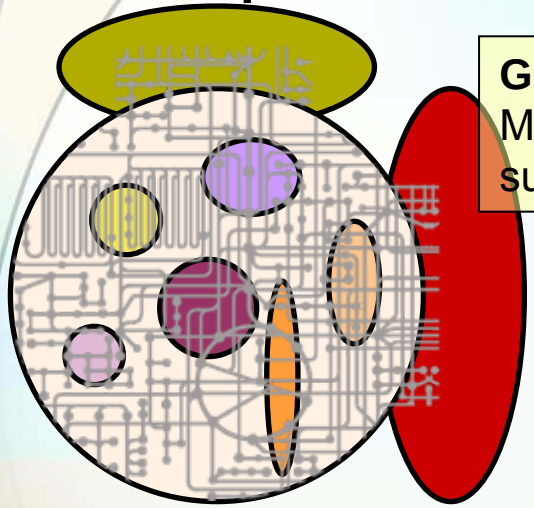
Evidence hierarchy

- Highest evidence:
  - Purification/characterization of enzymes
  - Marked substrates flow
  - Phenotypes by genetic variance
- Other sources of evidence:
  - Human genome
  - Transcriptomics data
  - Metabolomics, proteomics
  - In vitro cell studies

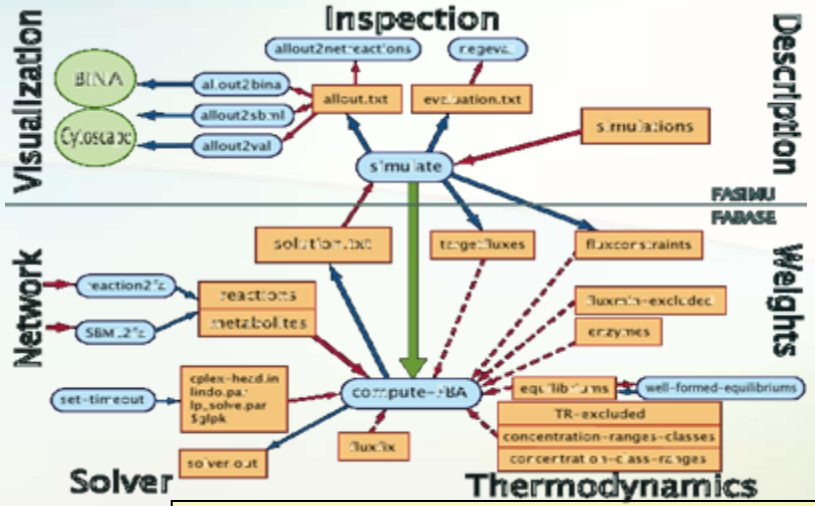
Parallel development

Data: **HepatoNet**



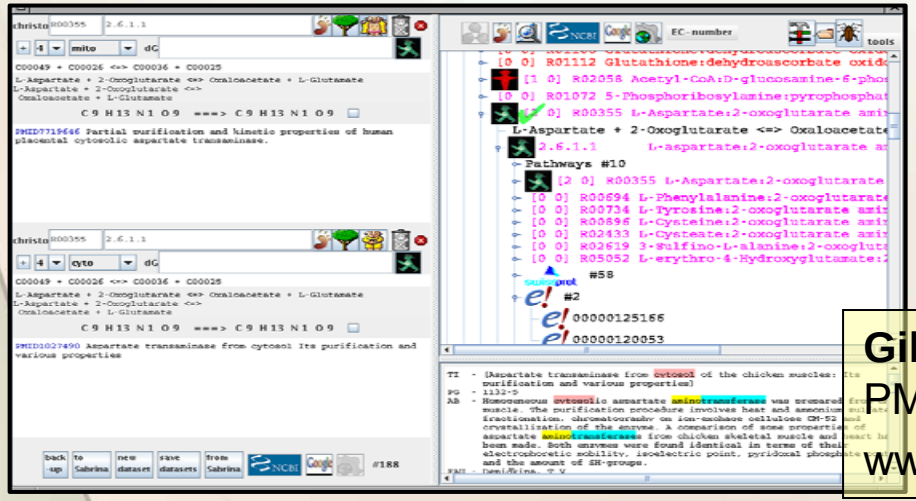
Gille et al.
Mol Sys Biol 2010
submitted

Computation framework: **FASIMU**



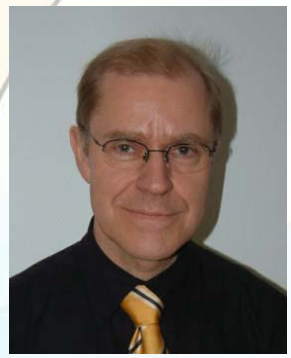
Hoppe et al. Bioinformatis 2010, submitted
www.bioinformatics.org/fasimu

Annotation software: **METANNOGEN**



Gille et al. BMC Syst Biol 2007
PMID 17408512
www.bioinformatics.org/strap/metannogen

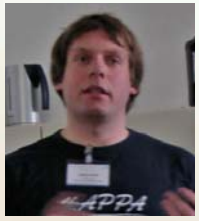
HepatoNet as a group‘s effort


Holzhütter: head, biophysics


Gille: medical biochemistry


Bölling: ontology


Hoppe: modelling


Bulik


Hoffmann

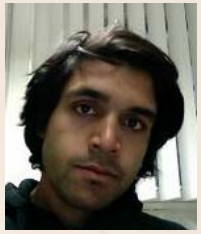

Hübner


Karlstädt

External contributors:


Behre


Gebhardt


Ganeshan


König

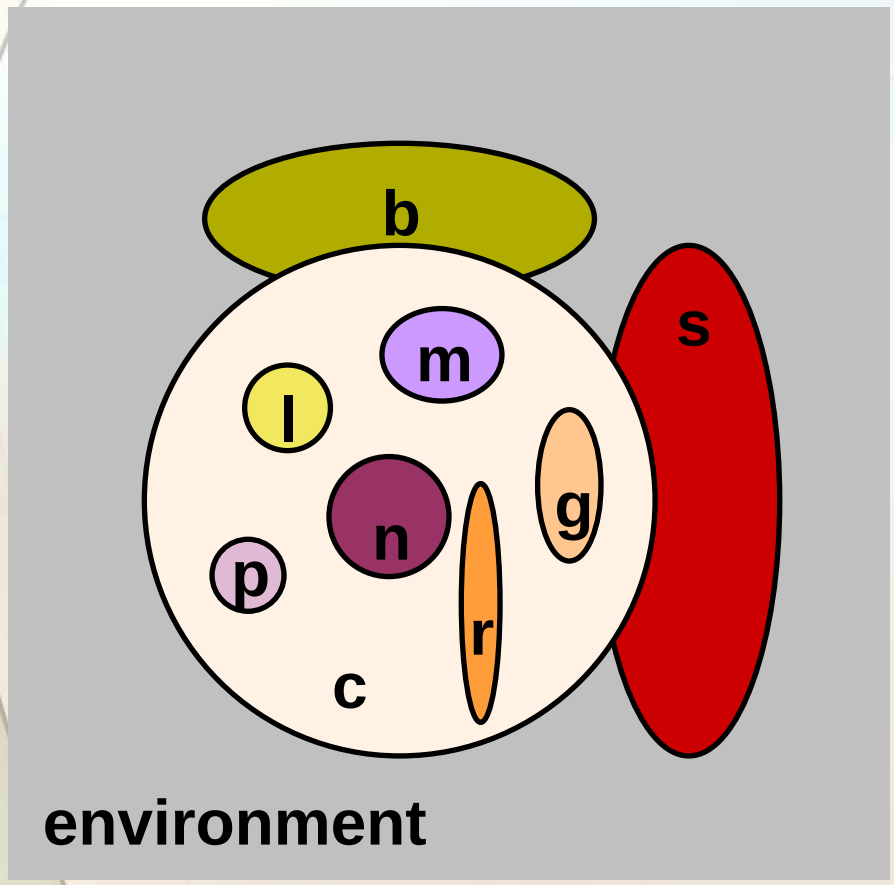

Rother


Weidlich


Handorf


Gevorgyan

HepatoNet reconstruction



2468 Reactions

Metabolic	1027
Transport	1441

1369 Compartment-specific metabolites

Cytosol [c]	600
Mitochondrion [m]	232
ER [r] & Golgi [g]	115
Peroxisome [p]	72
Lysosome [l]	65
Nucleus [n]	7
Plasma [s]	255
Bile [b]	23

Network before pruning

Reactions	3300
Metabolites	2412

Network reconstruction

Datasets evaluated	3342
Original references	2065

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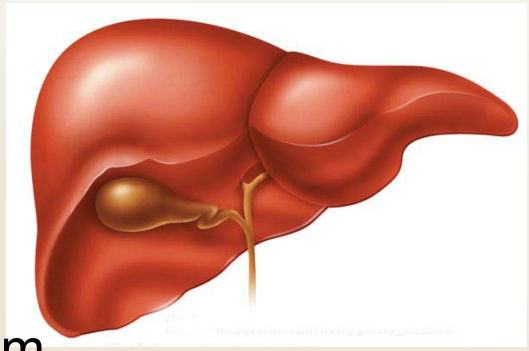
O_2 demand of NH_3 detoxification

- Important liver function
- Calculate flux distribution for conversion of NH_3 into specified fractions of urea (x), glutamine (y) and alanine (z)



NH_3

- intestinal bacteria
- amino acid metabolism

x → **Urea**

y → **Glutamine**

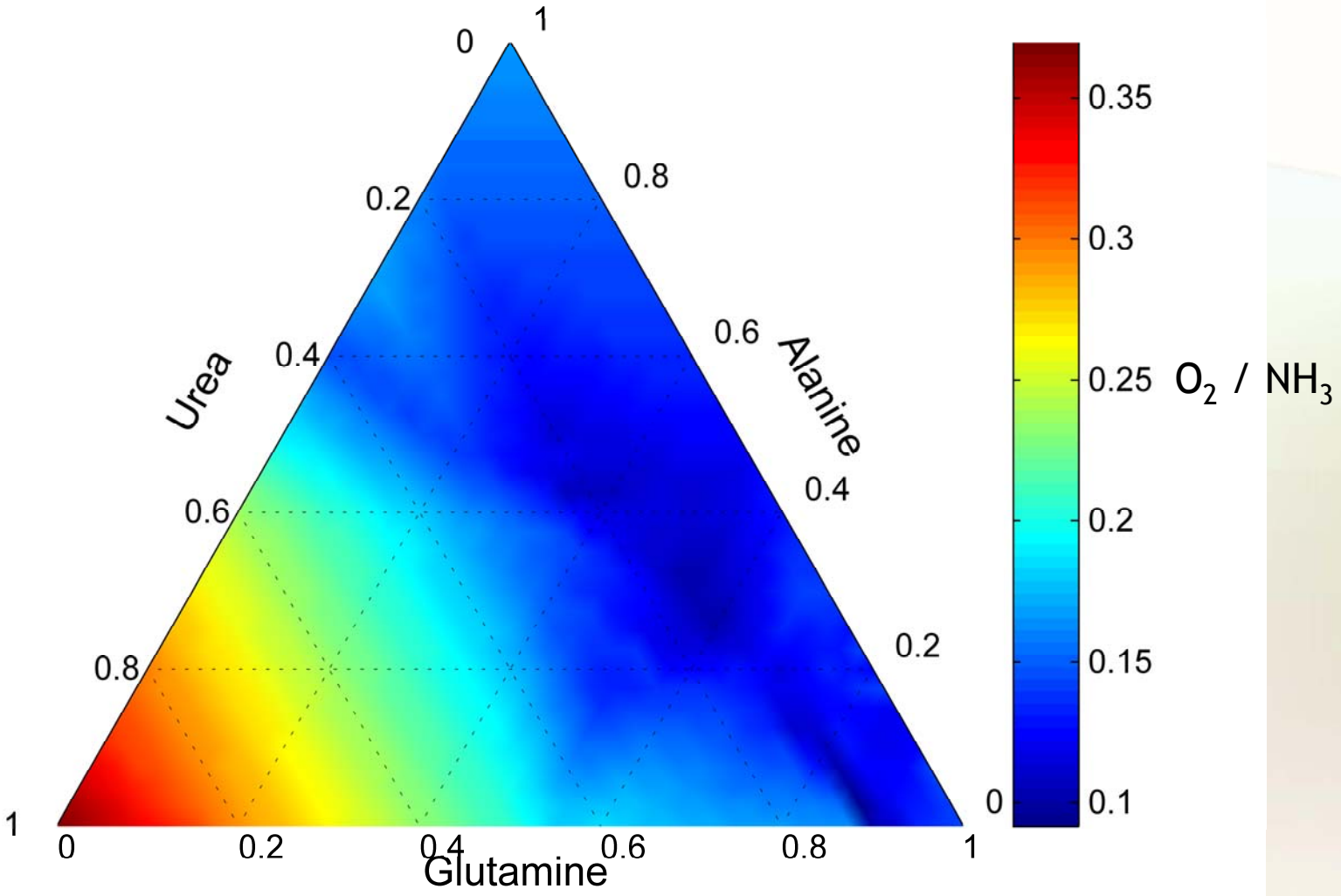
z → **Alanine**

Flux-balance optimization

- Steady state condition
- Flux minimization
- Thermodynamics constraint based on metabolite concentration ranges
  - Hoppe et al. BMC Syst Biol 2007, PMID 17543097

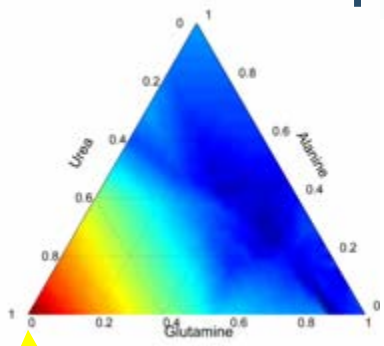
O_2 demand of NH_3 detoxification

- Predicted oxygen demand for NH3-detoxification

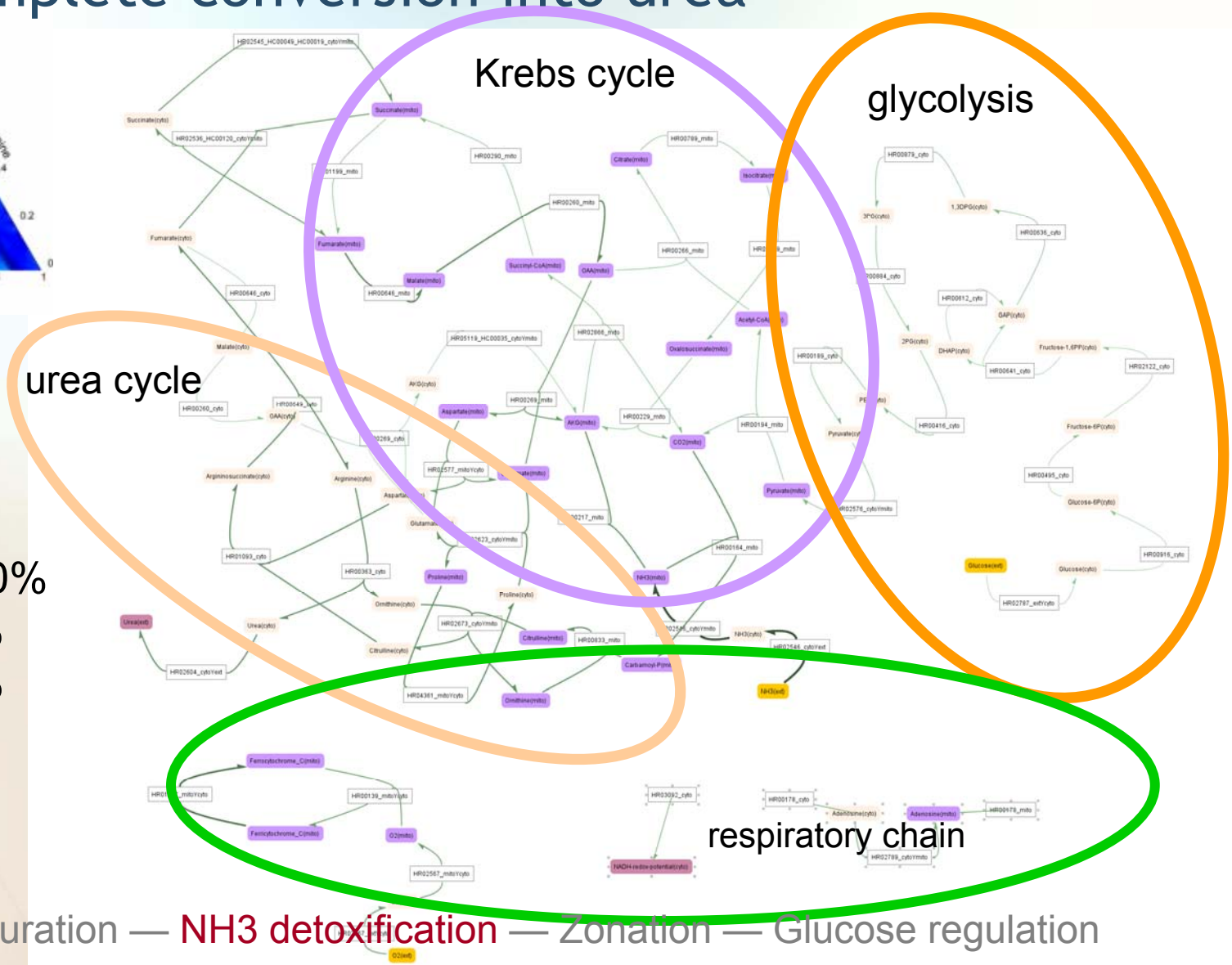


O_2 demand of NH_3 detoxification

- complete conversion into urea

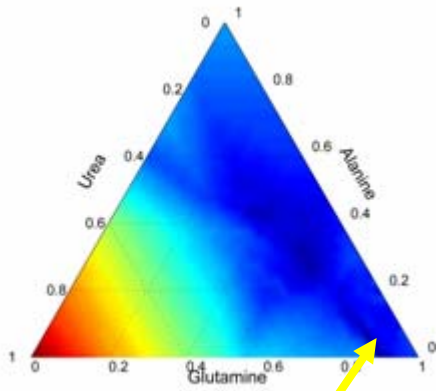


Urea = 100%
Ala = 0%
GlN = 0%



O_2 demand of NH_3 detoxification

- preferential conversion into glutamine



Urea = 10%
Ala = 2 %
Gln = 88%

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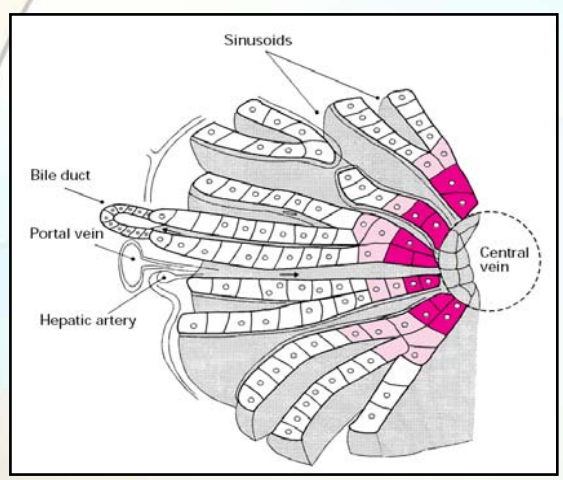
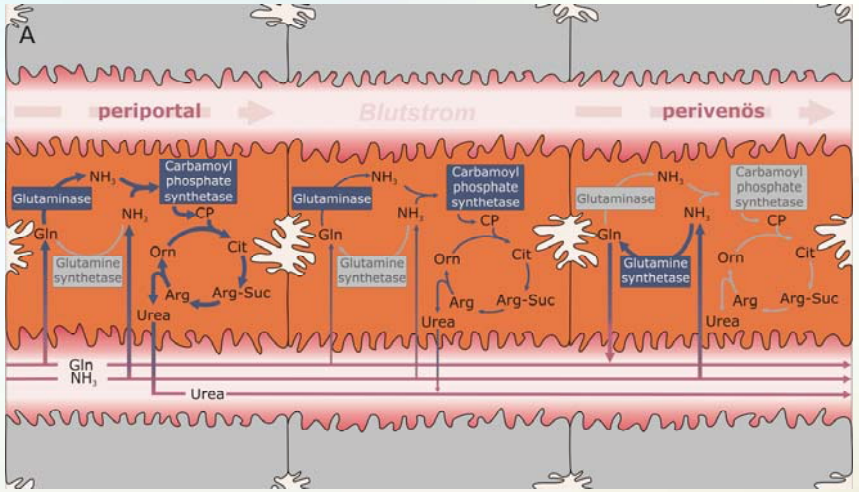
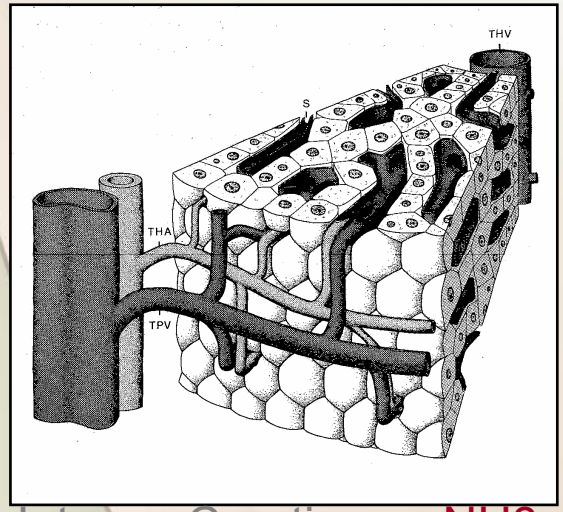
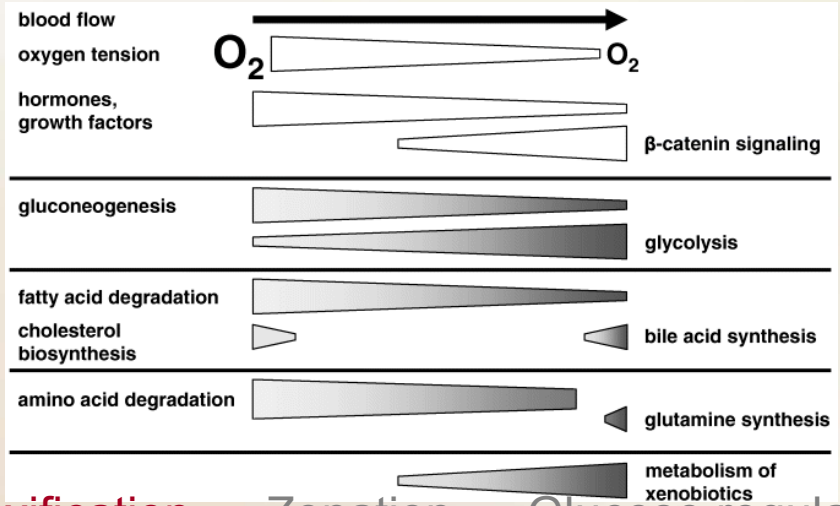
Functional heterogeneity along sinusoids





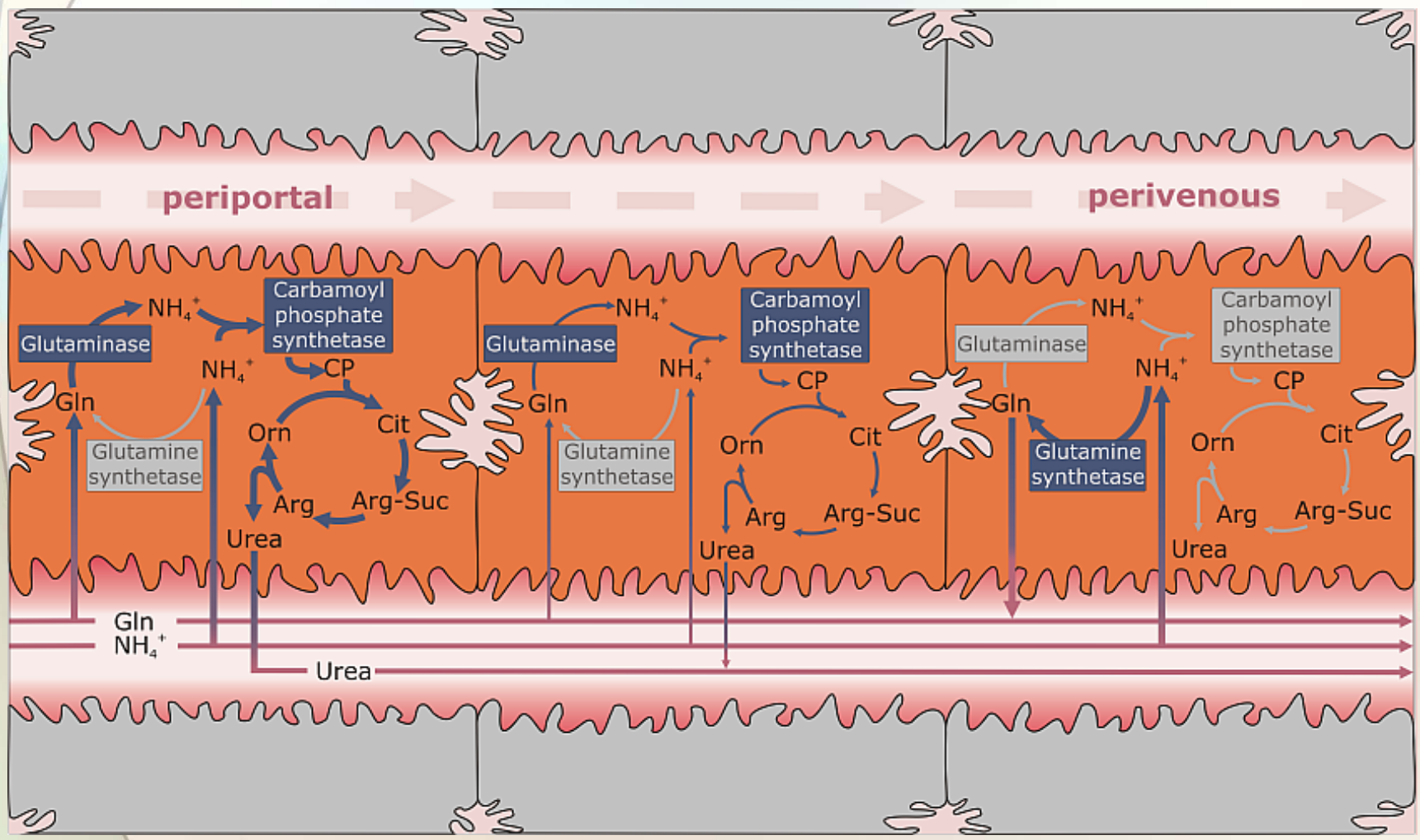




Modeling zonation

- Control parameters
  - O_2 gradient
  - Expression of amino acid transporters
- FBA correctly predicts
  - Periportal urea formation
  - Glutamine
    - imported by periportal hepatocytes
    - exported by perivenous hepatocytes

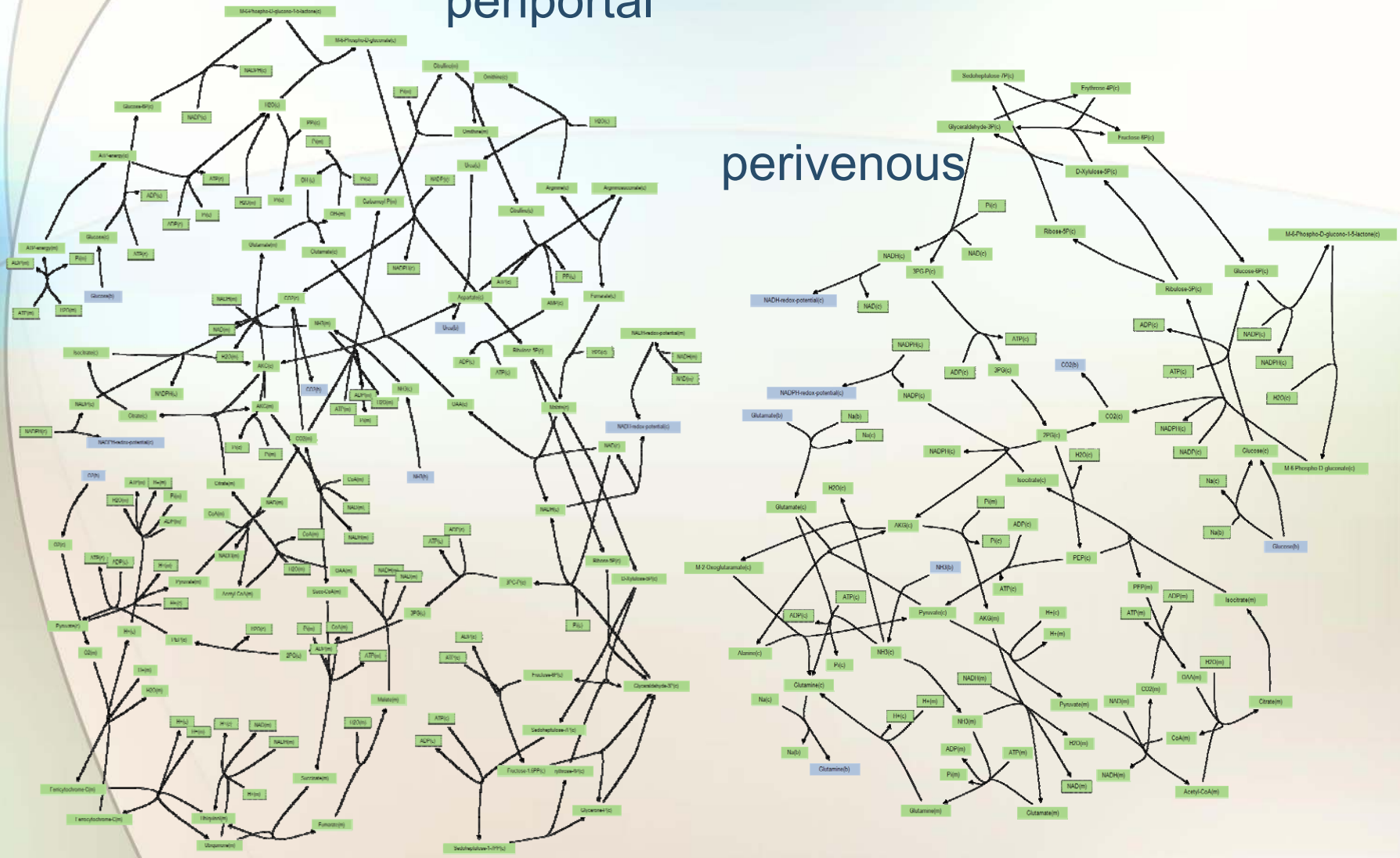
Modeling zonation



Modeling zonation - fluxes

periportal

perivenous



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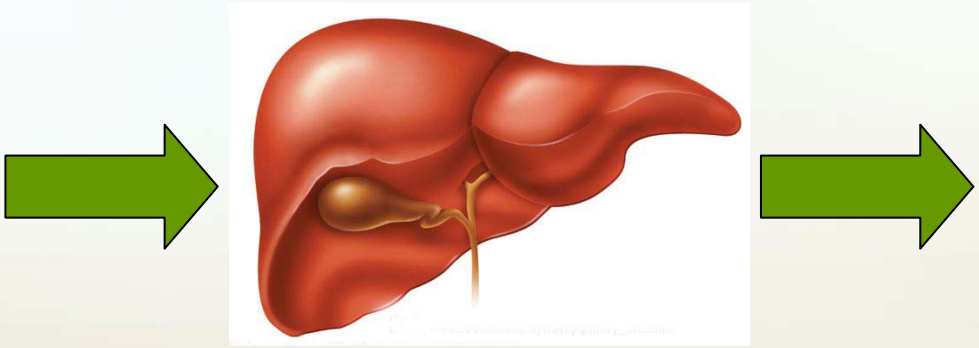
Hepatic regulation of blood glucose level

- crucial liver function



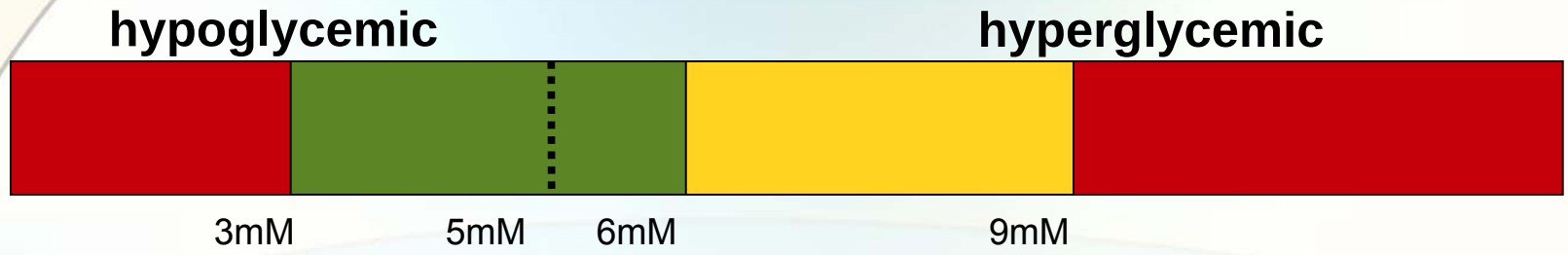
hepatic glucose utilization (HGU)

- Glycogen storage
- Glycolysis

hepatic glucose production (HGP)

- Glycogenolysis
- Gluconeogenesis

Blood glucose level is tightly controlled



Glucose shortage
most critical: brain

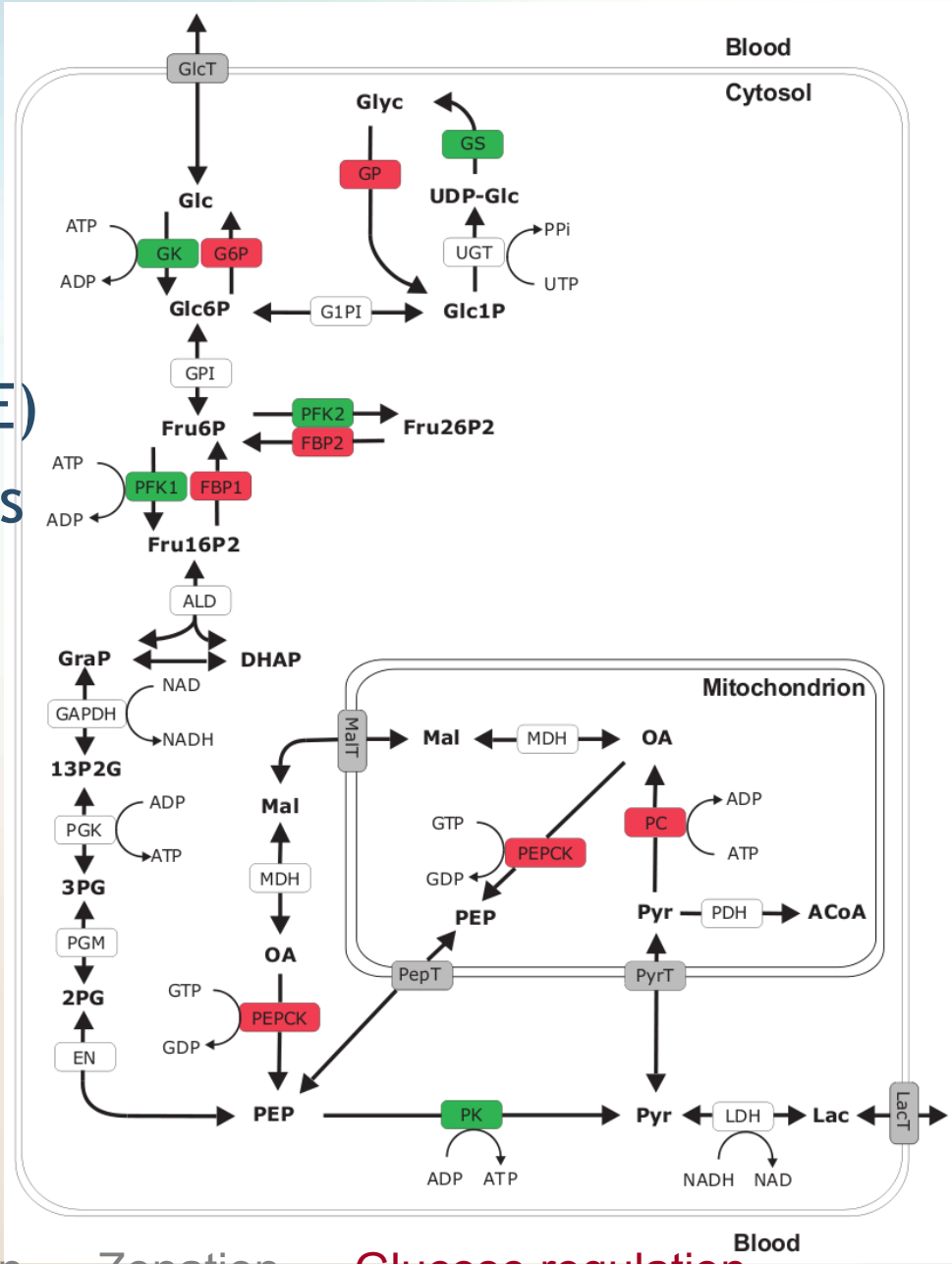
Glucose toxicity
protein modifications

	Overnight fast (12–16 h)	Moderate fast (30–60 h)	Prolonged fast (> 1 week)
*Overall glucose production**	10(100)	7.5(100)	5.0(100)
Hepatic output	10(100)	7.1(95)	4.5(90)
Glycogenolysis	5.0(50)	0.4(15)	0(0)
Gluconeogenesis	5.0(50)	6.7(90)	4.5(90)
Renal gluconeogenesis	0(0)	0.3(5)	0.5(10)
*Overall glucose utilization**	10(100)	7.5(100)	5.0(100)
Brain	5.0(50)	4.4(60)	3.5(70)
Splanchnic tissues	1.5(15)	0.9(12)	0.3(6)
Muscle	1.5(15)	0.8(10)	0.3(6)
Blood cells, skin	1.0(10)	0.8(10)	0.6(12)
Renal medulla	0.5(5)	0.4(5)	0.2(4)
Adipose tissue	0.5(5)	0.2(3)	0.1(2)

* μmol/kg per min (percent of total).

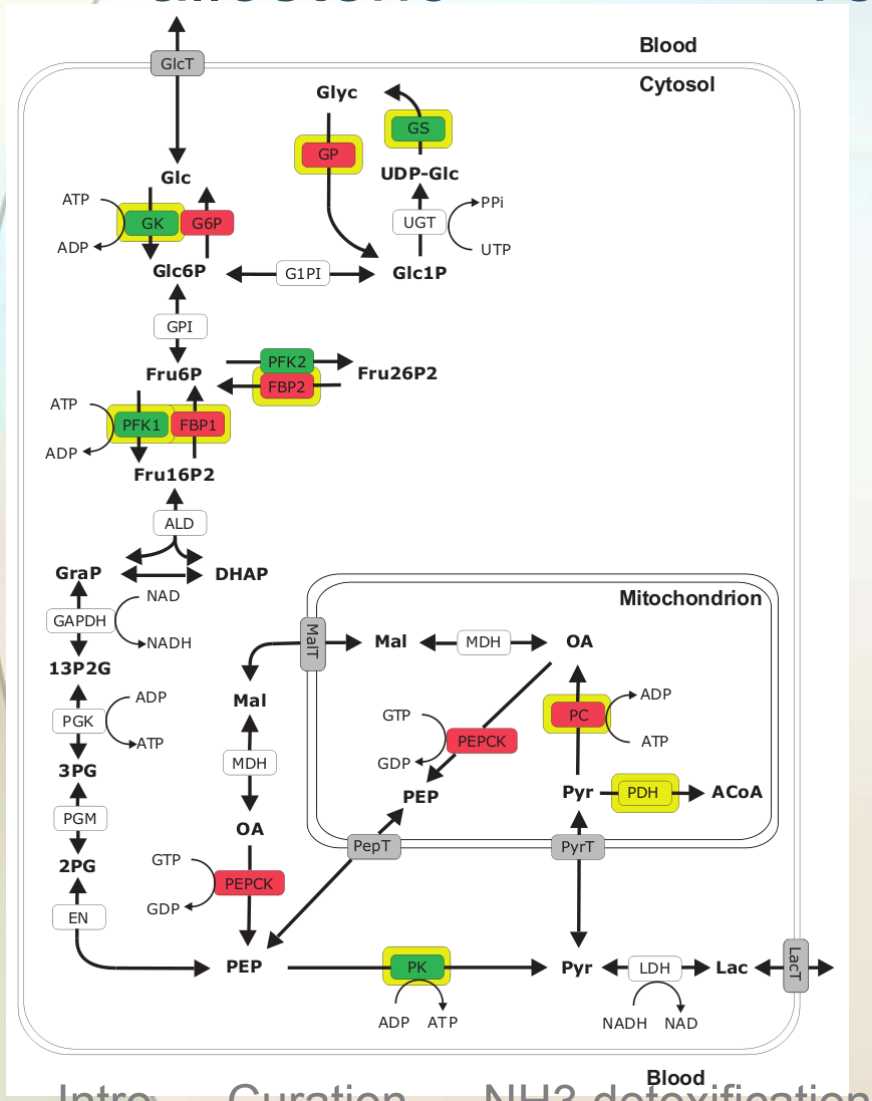
Kinetic model

- Glycolysis, gluconeogenesis and glycogen metabolism
- Detailed kinetic model (ODE)
- 36 reactions, 49 metabolites
- 3 compartments
- Kinetics specific for human hepatocyte
- Switch between **HGP** and **HGU** regulated by different mechanisms on different time scales

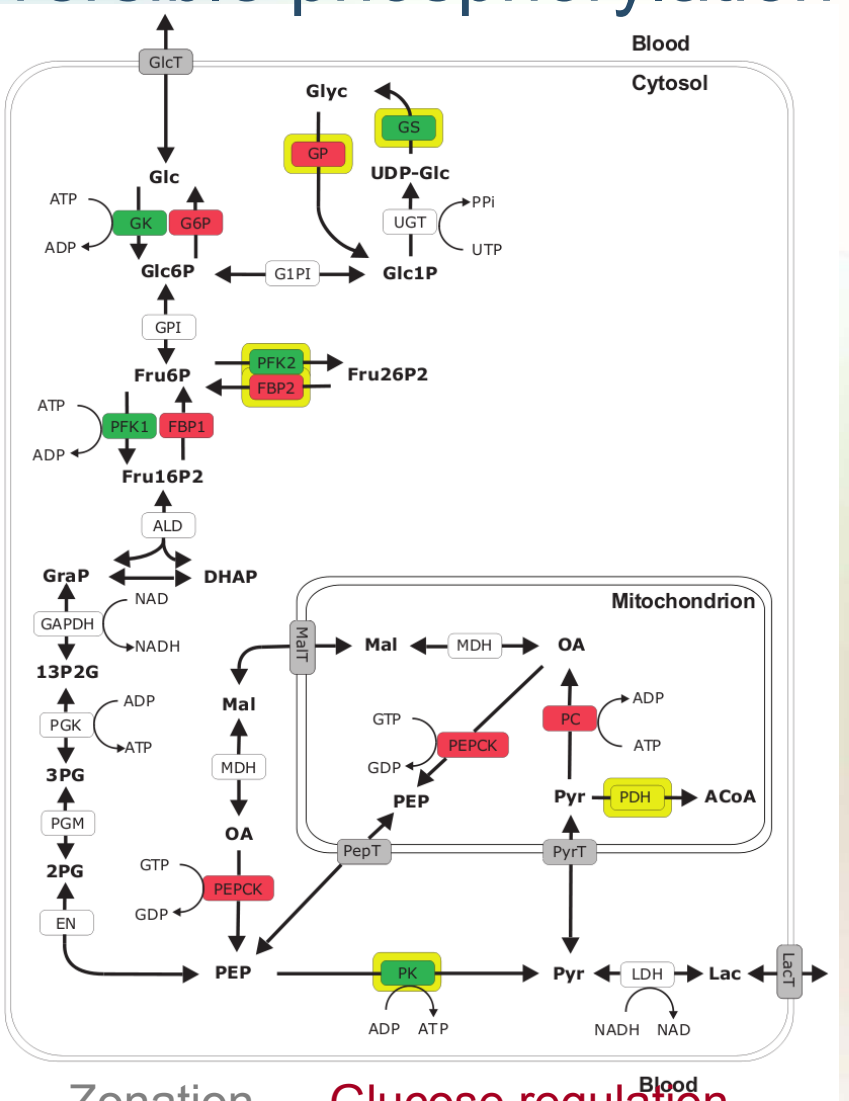


Fast regulation

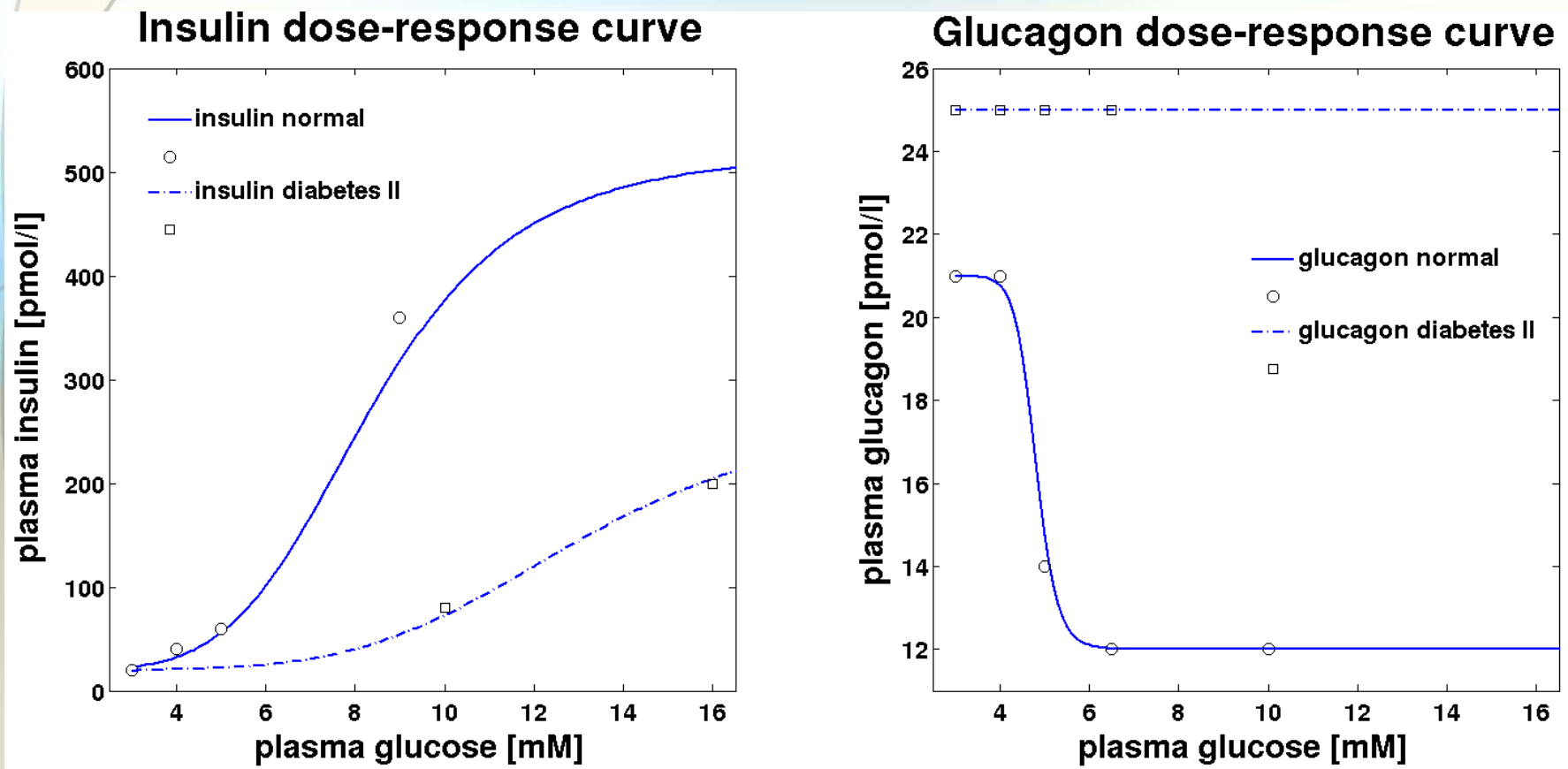
allosteric



reversible phosphorylation

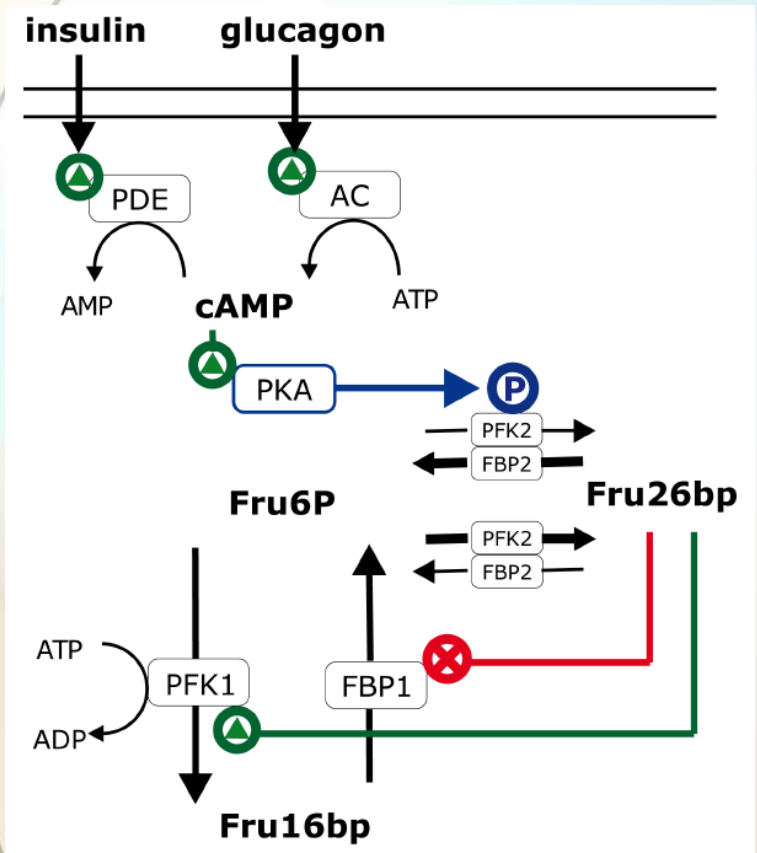


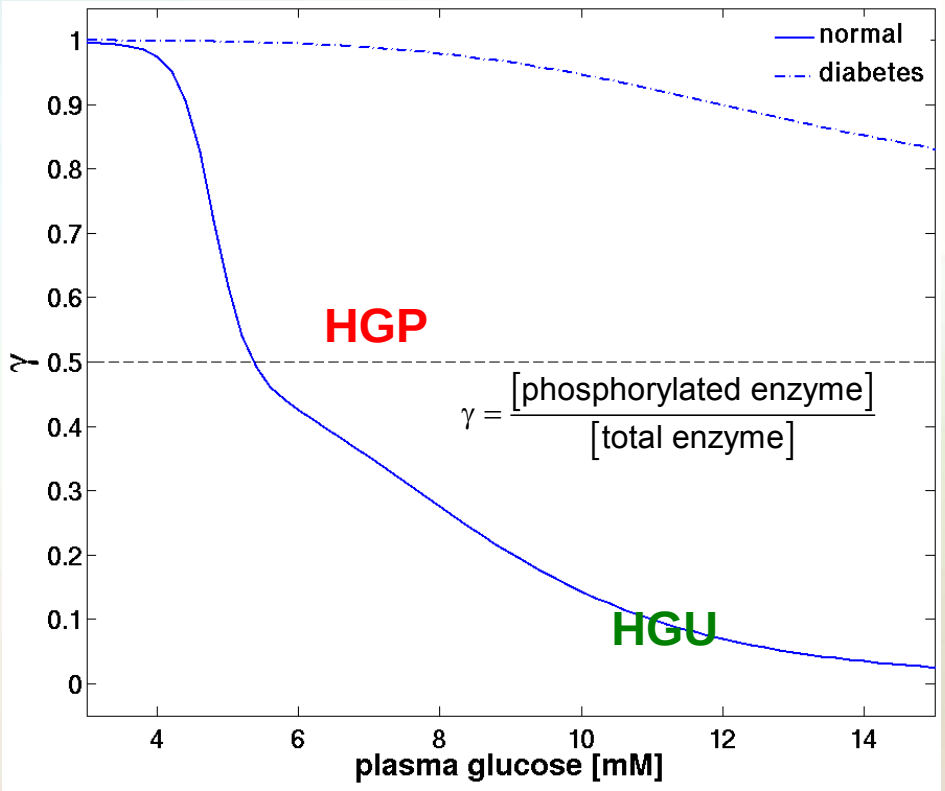
Regulation by insulin & glucagon



Counterregulatory hormones changing with plasma glucose

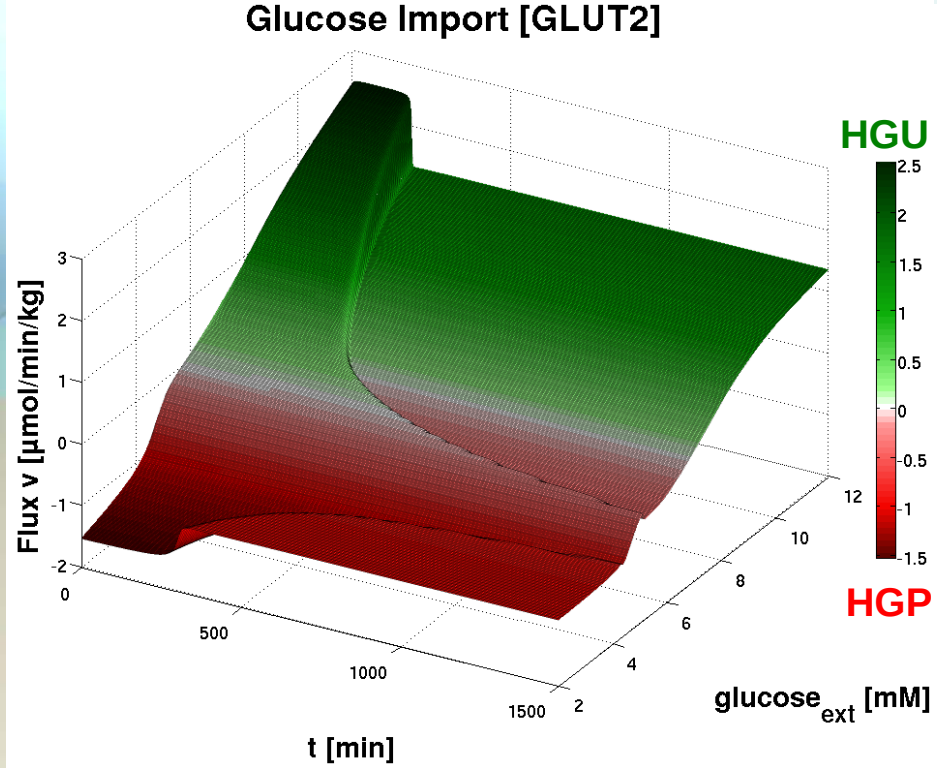
Regulation by insulin & glucagon

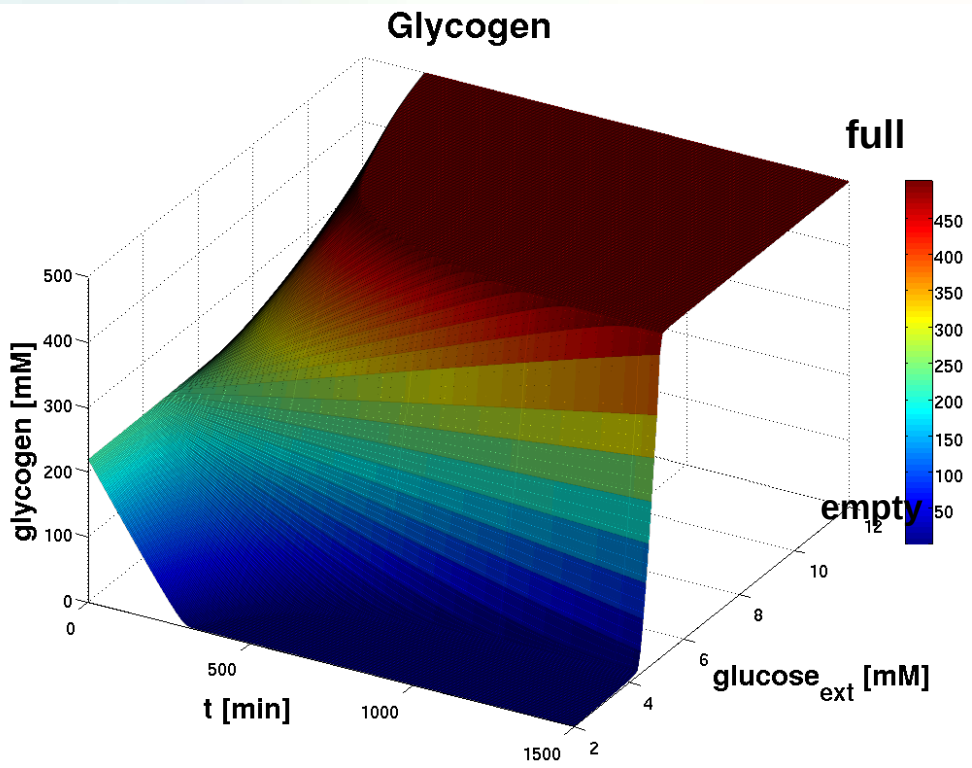




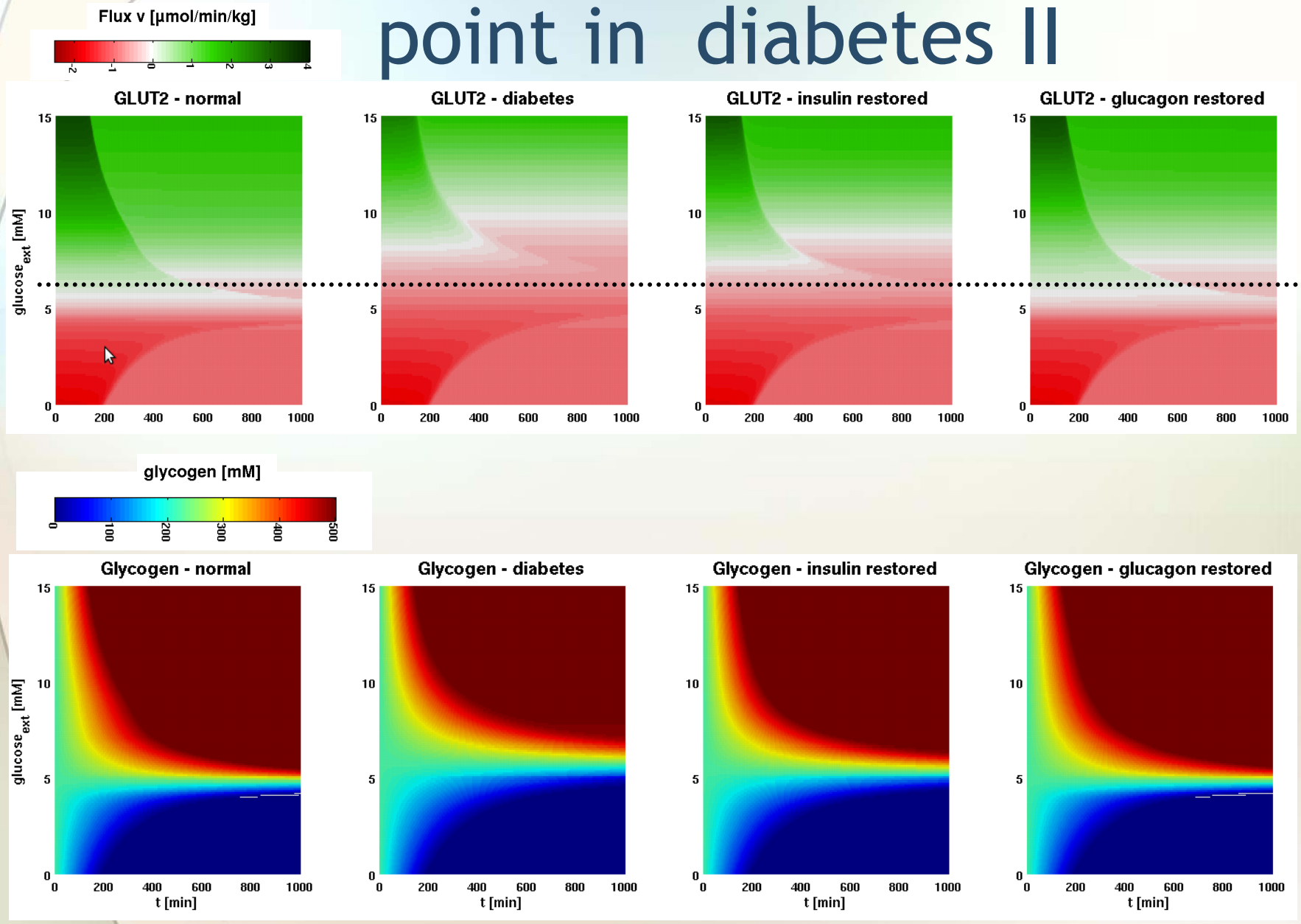
Insulin and glucagon determine the phosphorylation state of interconvertible enzymes

Simulation of temporal HPG/HPU switch





Shift of HPG-HPU switch point in diabetes II



Take-home message

- Metabolic networks require manual curation
- HepatoNet ready for flux-balance calculations
  - O_2 demand of NH_3 detoxification
  - Zonation
- HepatoNet resource for kinetic models
  - Regulation of blood glucose

The end

- Thanks for the attention!

Groups website:
www.charite.de/sysbio