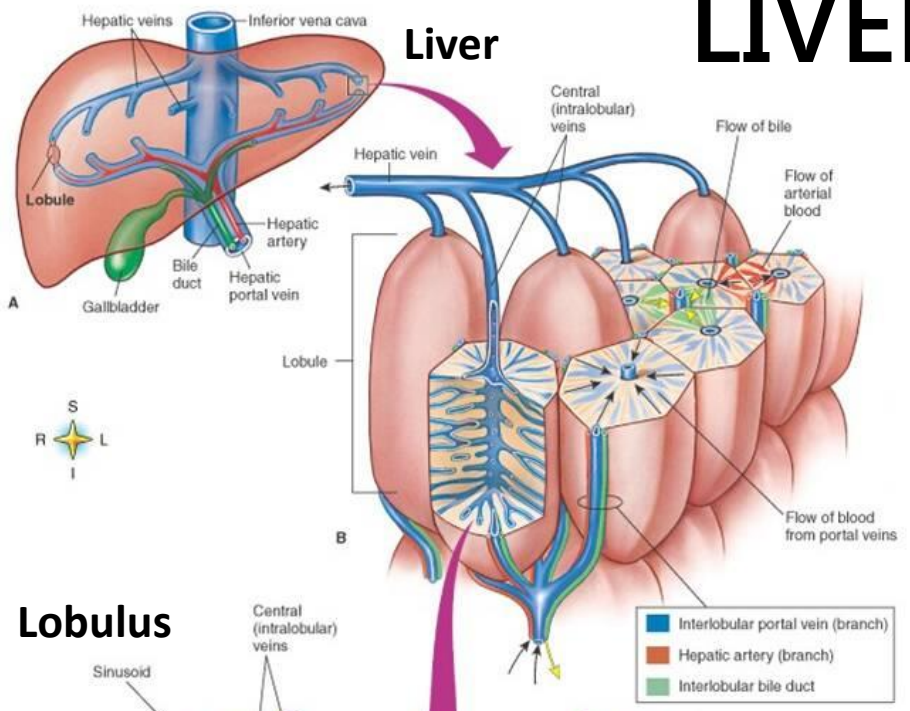
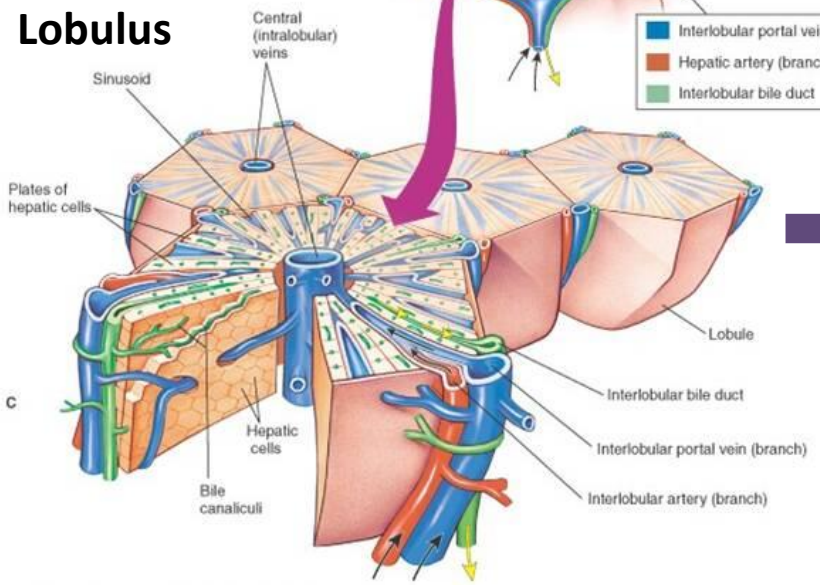
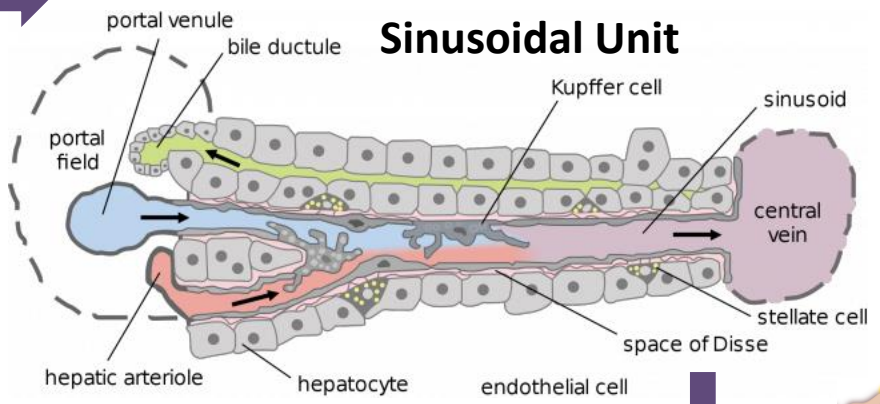
LIVER ARCHITECTURE IN A NUTSHELL

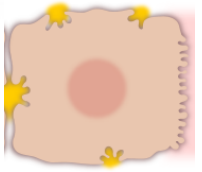




- liver structured in parallel subunits (**liver lobulus**)
- lobulus consists of **network of sinusoids** connecting hepatic artery & portal vein with the **central vene (periportal → perivenous)**
- **Sinusoidal Unit**
  central blood vessel (sinusoid) surrounded by hepatocytes

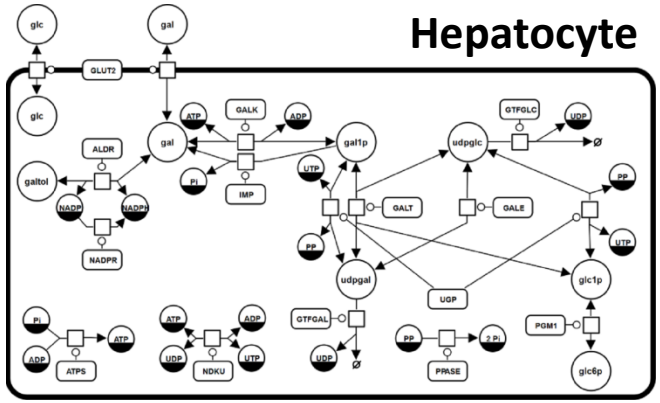


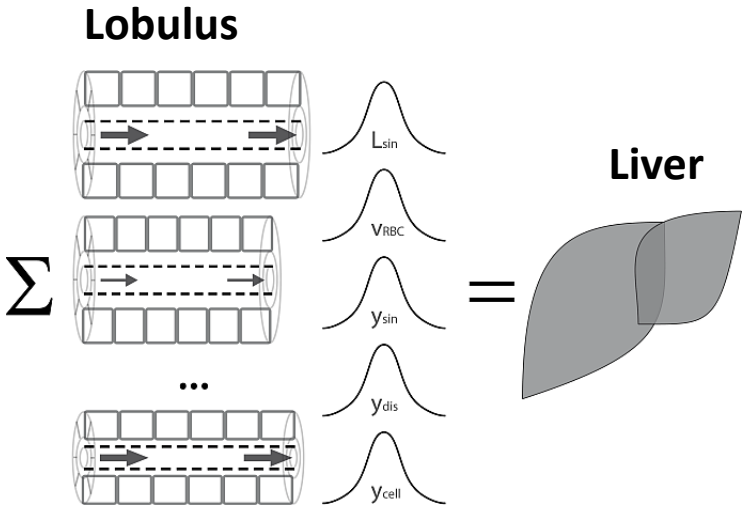
Hepatocyte

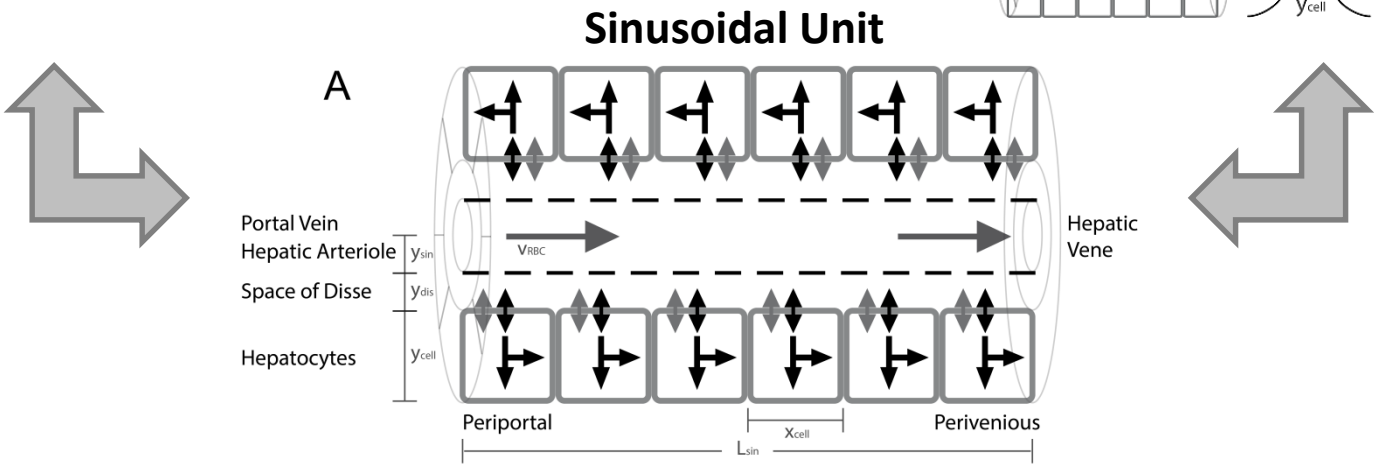


From Hepatocytes to Whole Liver Function: A Multi-scale Model of Human Galactose Metabolism

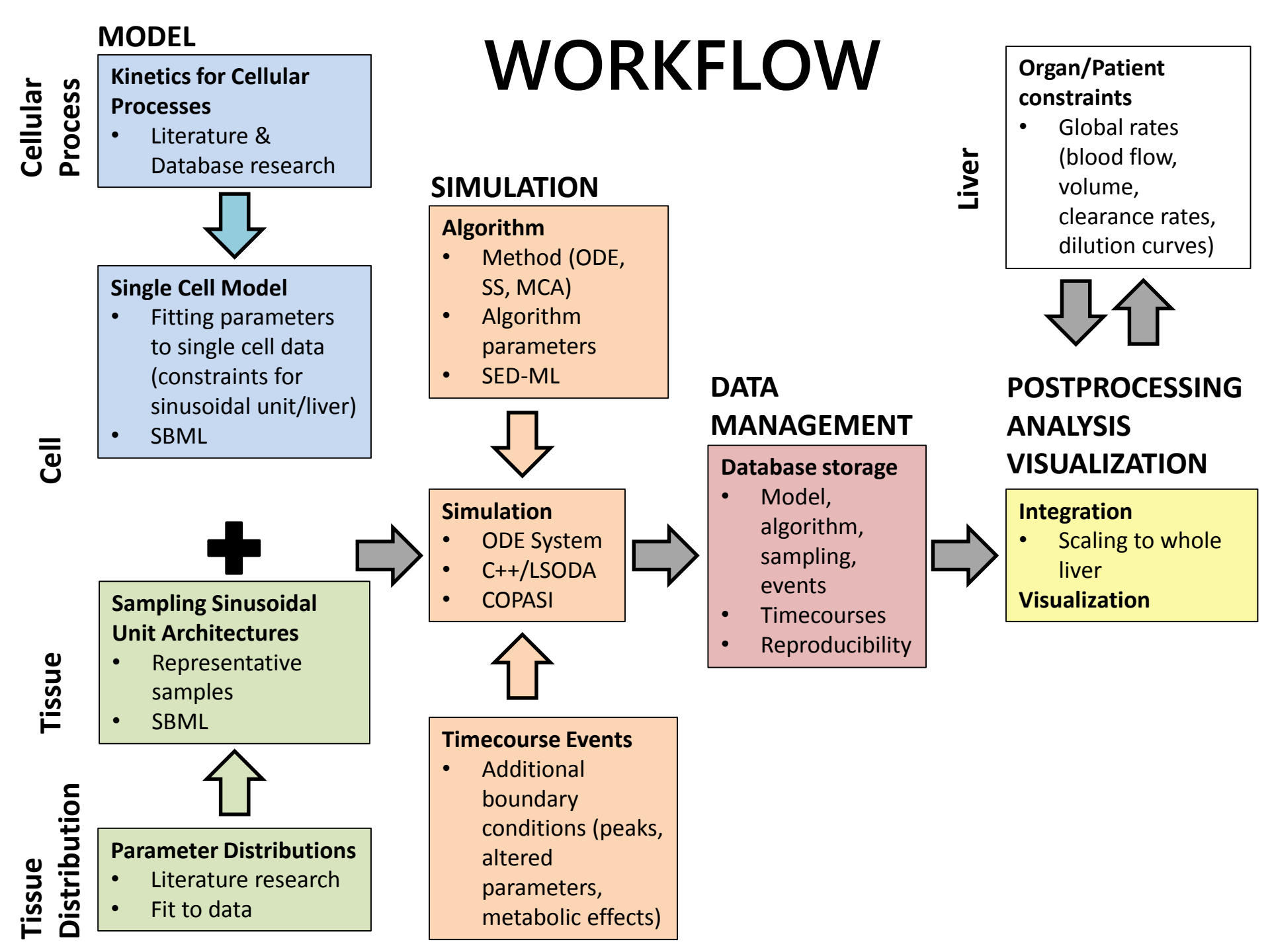
König M. & Holzhütter HG.





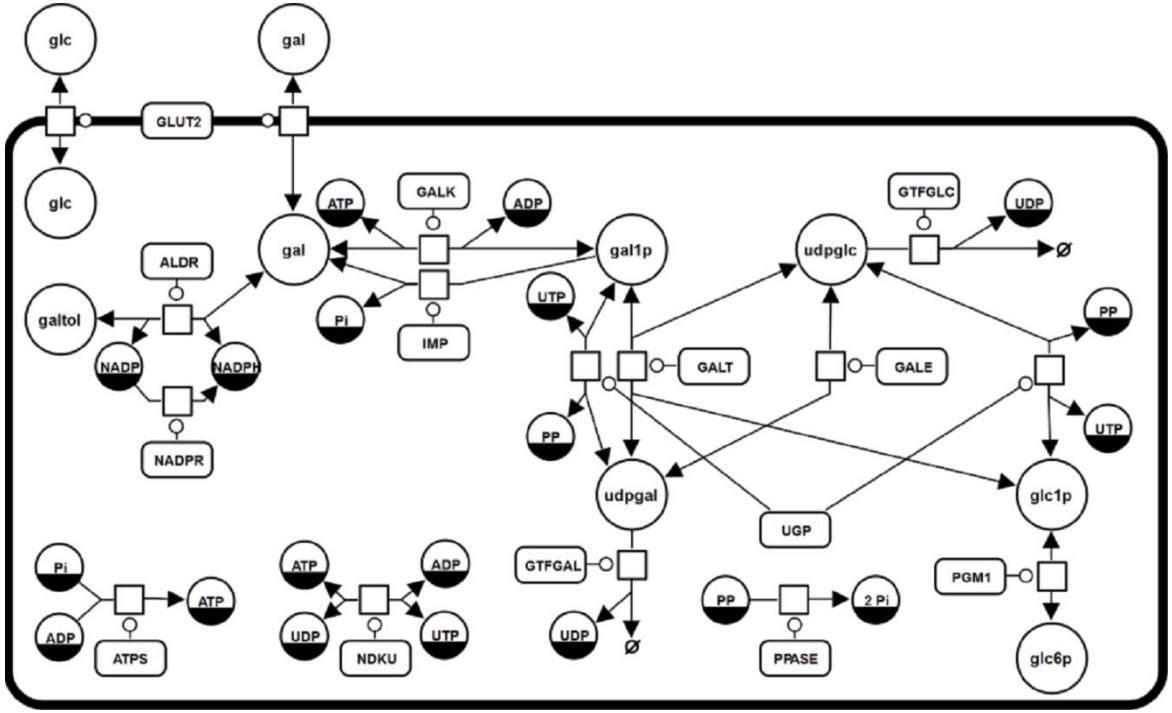


WORKFLOW


MODEL
Cellular Process
Kinetics for Cellular Processes
• Literature & Database research
Cell
Single Cell Model
• Fitting parameters to single cell data (constraints for sinusoidal unit/liver)
• SBML
Tissue
Sampling Sinusoidal Unit Architectures
• Representative samples
• SBML
Tissue Distribution
Parameter Distributions
• Literature research
• Fit to data
SIMULATION
Algorithm
• Method (ODE, SS, MCA)
• Algorithm parameters
• SED-ML
Simulation
• ODE System
• C++/LSODA
• COPASI
Timecourse Events
• Additional boundary conditions (peaks, altered parameters, metabolic effects)
DATA MANAGEMENT
Database storage
• Model, algorithm, sampling, events
• Timecourses
• Reproducibility
Liver
Organ/Patient constraints
• Global rates (blood flow, volume, clearance rates, dilution curves)
POSTPROCESSING
ANALYSIS
VISUALIZATION
Integration
• Scaling to whole liver
Visualization

CELLULAR GALACTOSE METABOLISM

- **liver most important organ for whole-body metabolism** and clearance of galactose **(>95%)**
- Galactose elimination capacity (GEC) **measurement for functional liver cell** mass
- Galactose clearance altered in most liver diseases (metabolic & structural)
  - Galactosemias
  - Cirrhosis
  - Hepatitis
  - Partial hepatectomy & regeneration
  - Aging
  - …

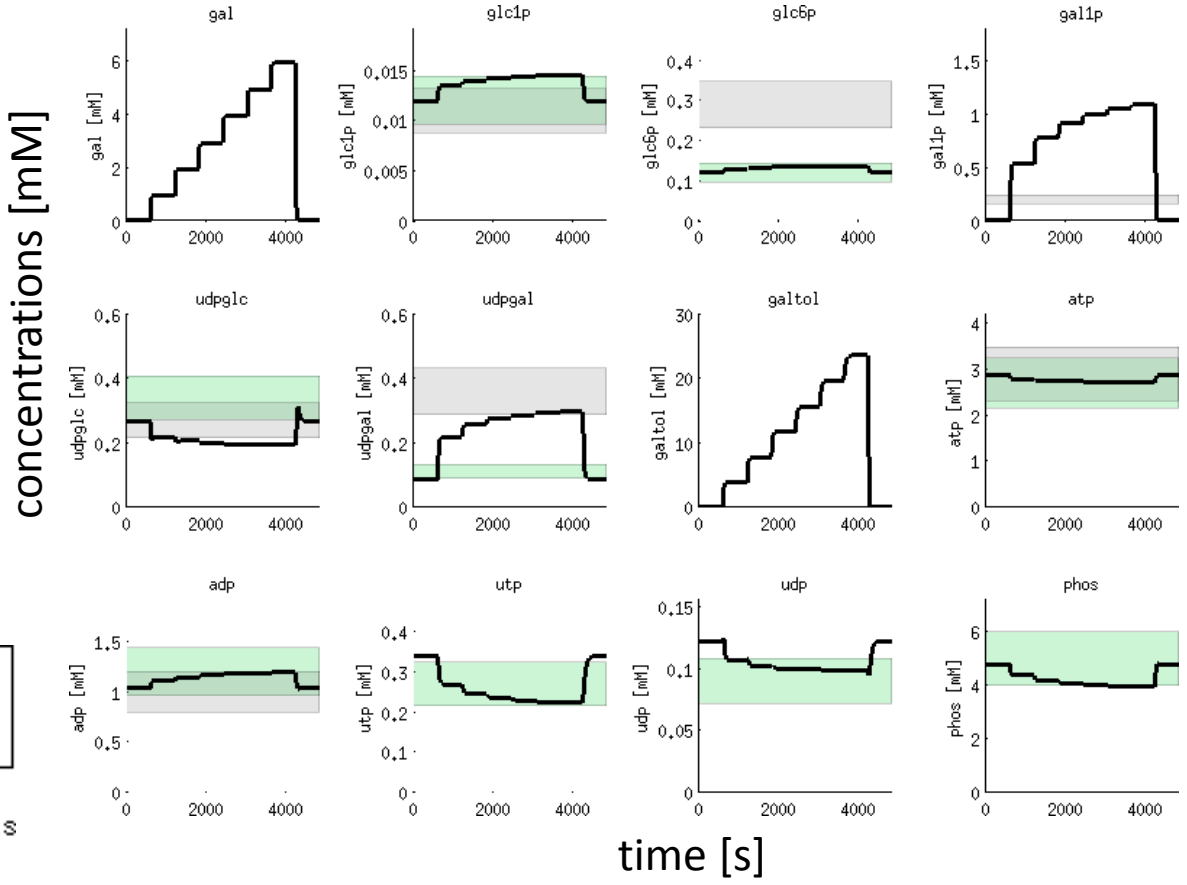


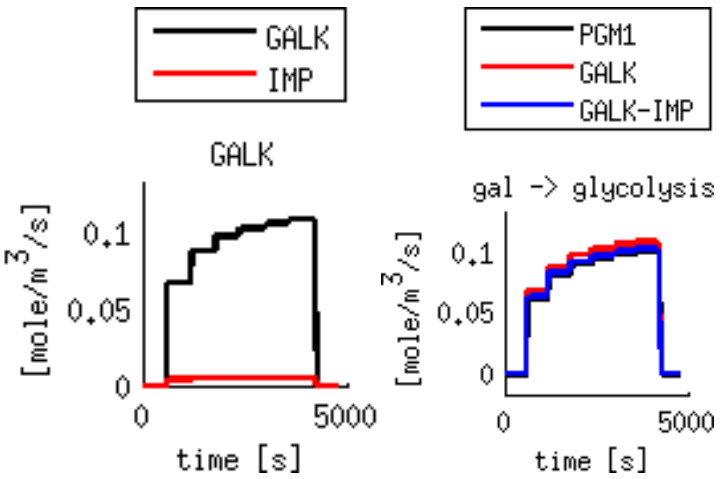
Detailed kinetic model

- ODEs for all processes involved in galactose metabolism
- SBML

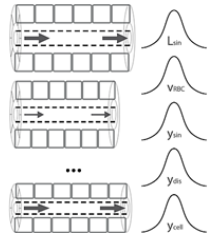
HEPATOCYTE MODEL

- **Reproduces**
  - concentrations normal state & in galactose challenge
  - saturation of galactose clearance
  - effects of galactosemias

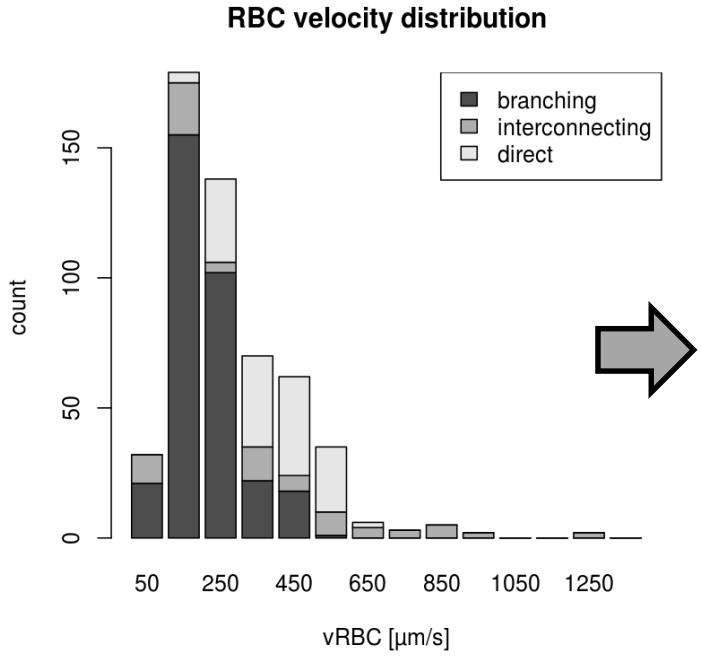


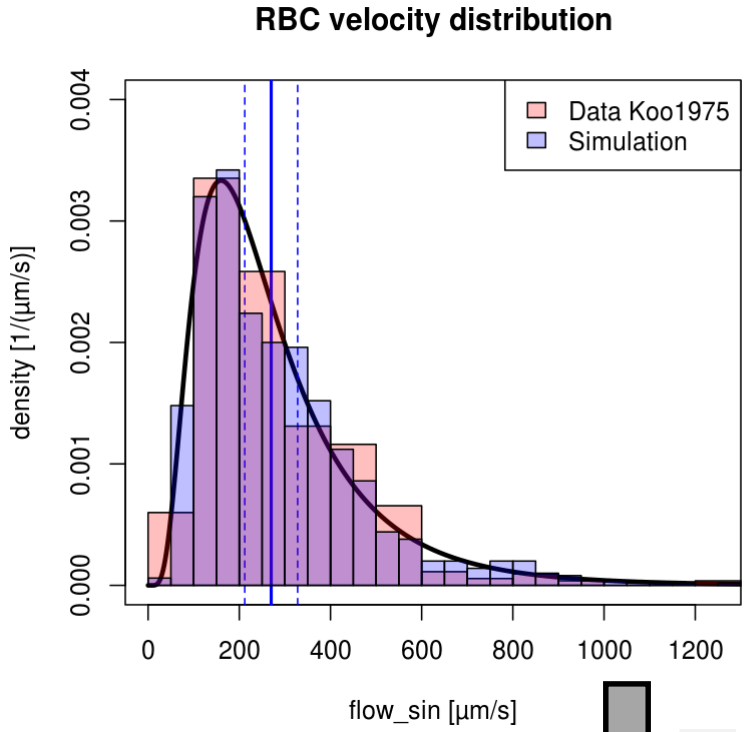


TISSUE DISTRIBUTIONS



- Fitting distribution of tissue parameters via maximum-likelihood methods

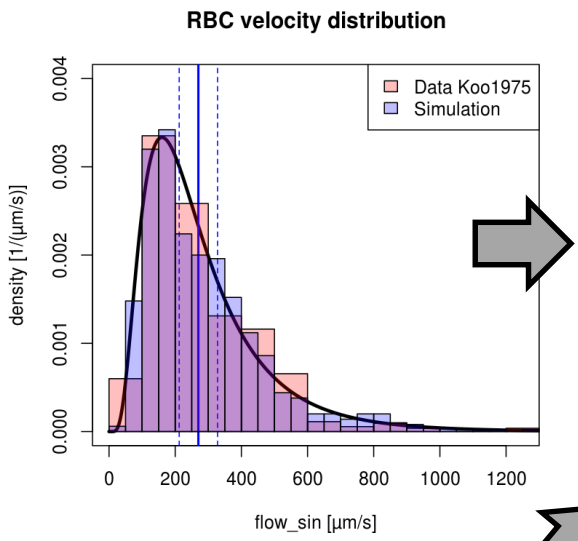


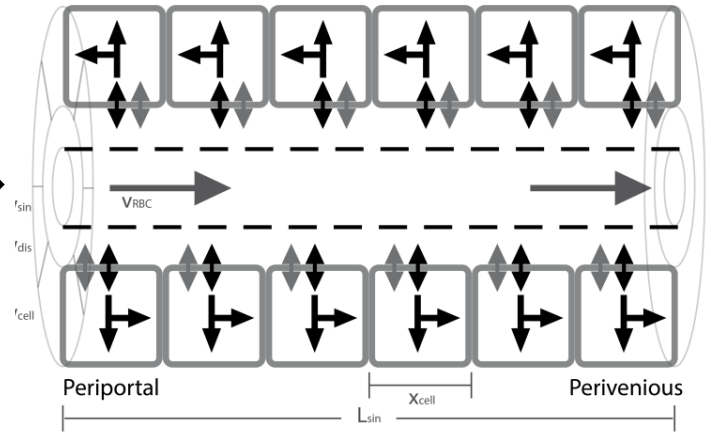


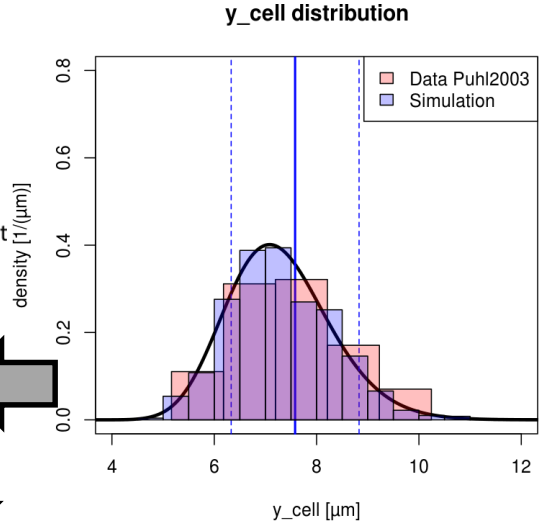
Parameter		meanlog	stdlog	mean μ (reported)	standard deviation (reported) SD	references
Sinusoidal length	L_{sin}	6.184	0.2462	500µm	125µm	based on distance between central veins **809±199µm** (SD, n=79, young rat, SEM of corrosion cast) (Warren, et al., 2008) scaled to human sinusoidal length
Sinusoidal radius	y_{sin}	1.465 (±0.010)	0.1017 (±0.0073)	4.4µm	0.45µm	Based on distribution of sinusoidal diameter **8.8±0.9µm** (SD, n=440 in N=11 human, OPS) (Puhl, et al., 2003)
Width of Disse space	y_{dis}	0.1296	0.3246	1.2µm	0.4µm	**0.4-1.5µm** (human, SEM, estimated from imaged) (Muto, et al., 1977) **0.5-1.2µm** (human, SEM, estimated from image) (Burwen, et al., 1982)
Hepatocyte sheet thickness	y_{cell}	1.977 (±0.014)	0.1390 (±0.0099)	7.58µm	1.25µm	**7.58µm** Calculated from functional sinusoidal density FSD FSD **391±30** [1/cm] (SD, n=88, human, OPS) (Puhl, et al., 2003)

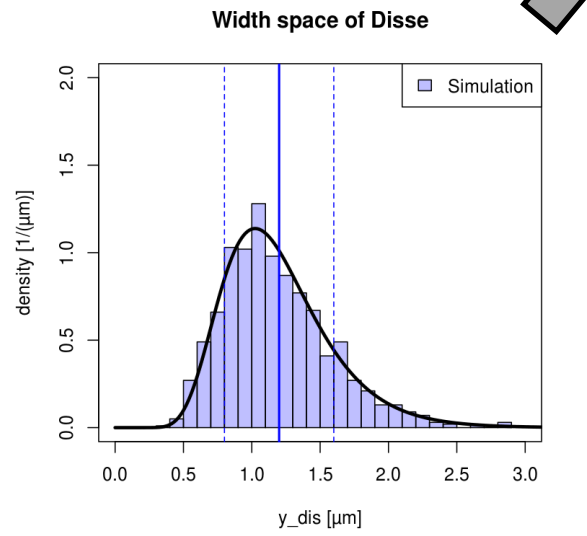
$$\frac{1}{}$$

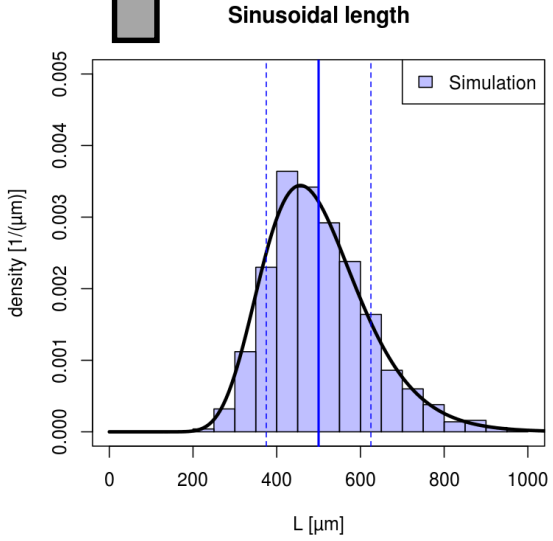
TISSUE-SCALE SINUSOIDAL UNIT

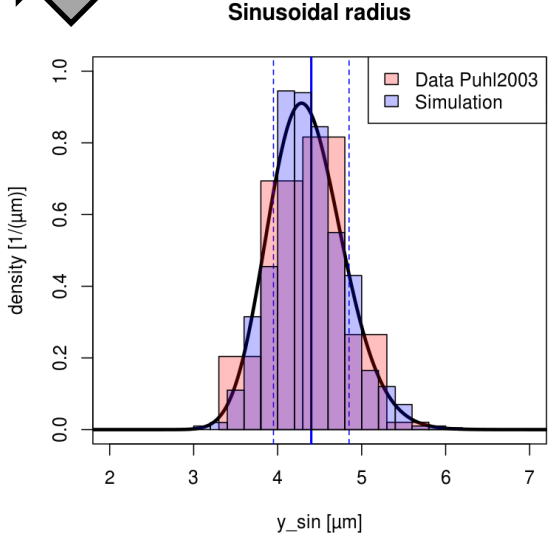




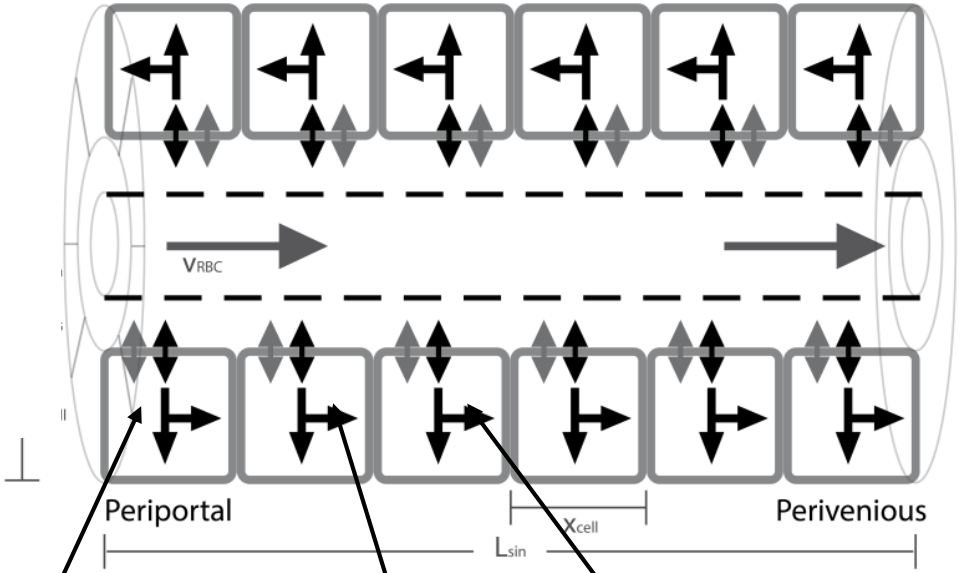


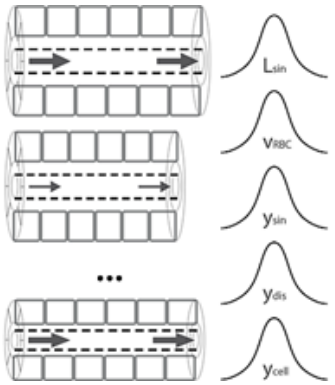


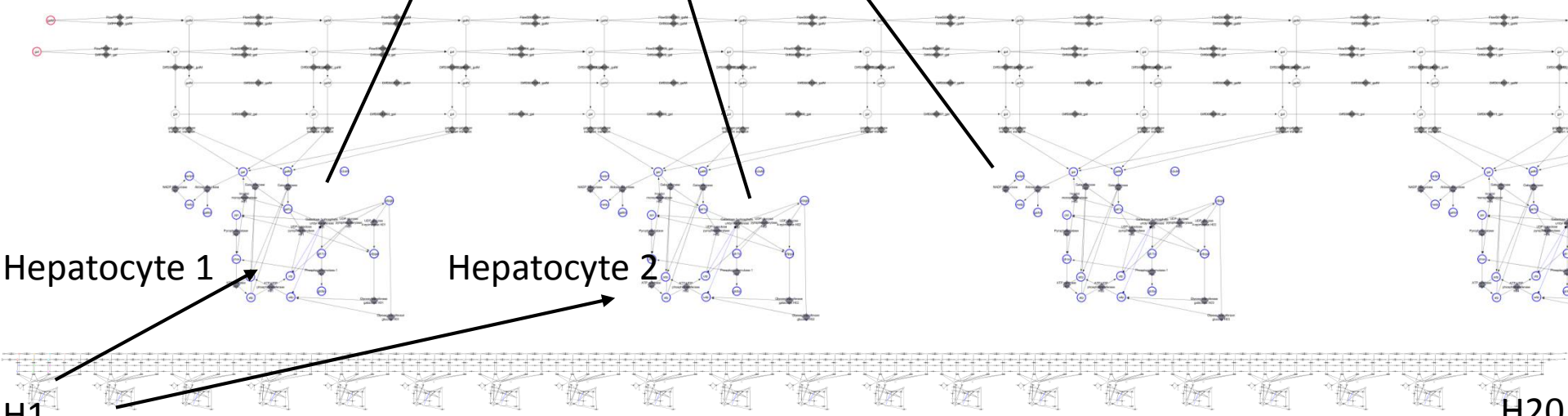




MULTITUDE OF LARGE-SCALE ODE MODELS

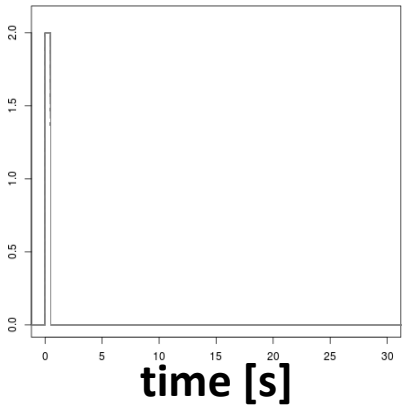


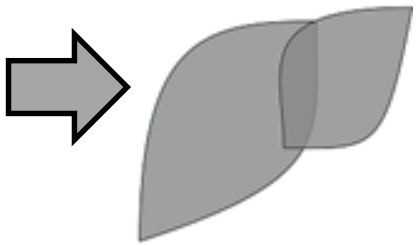


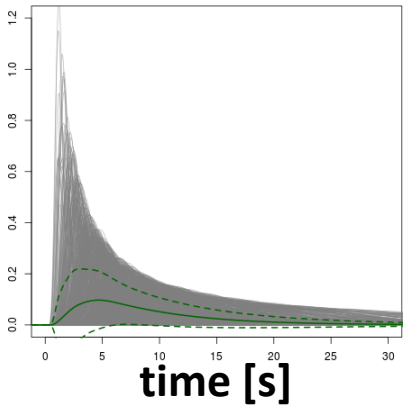


MULTIPLE INDICATOR DILUTION

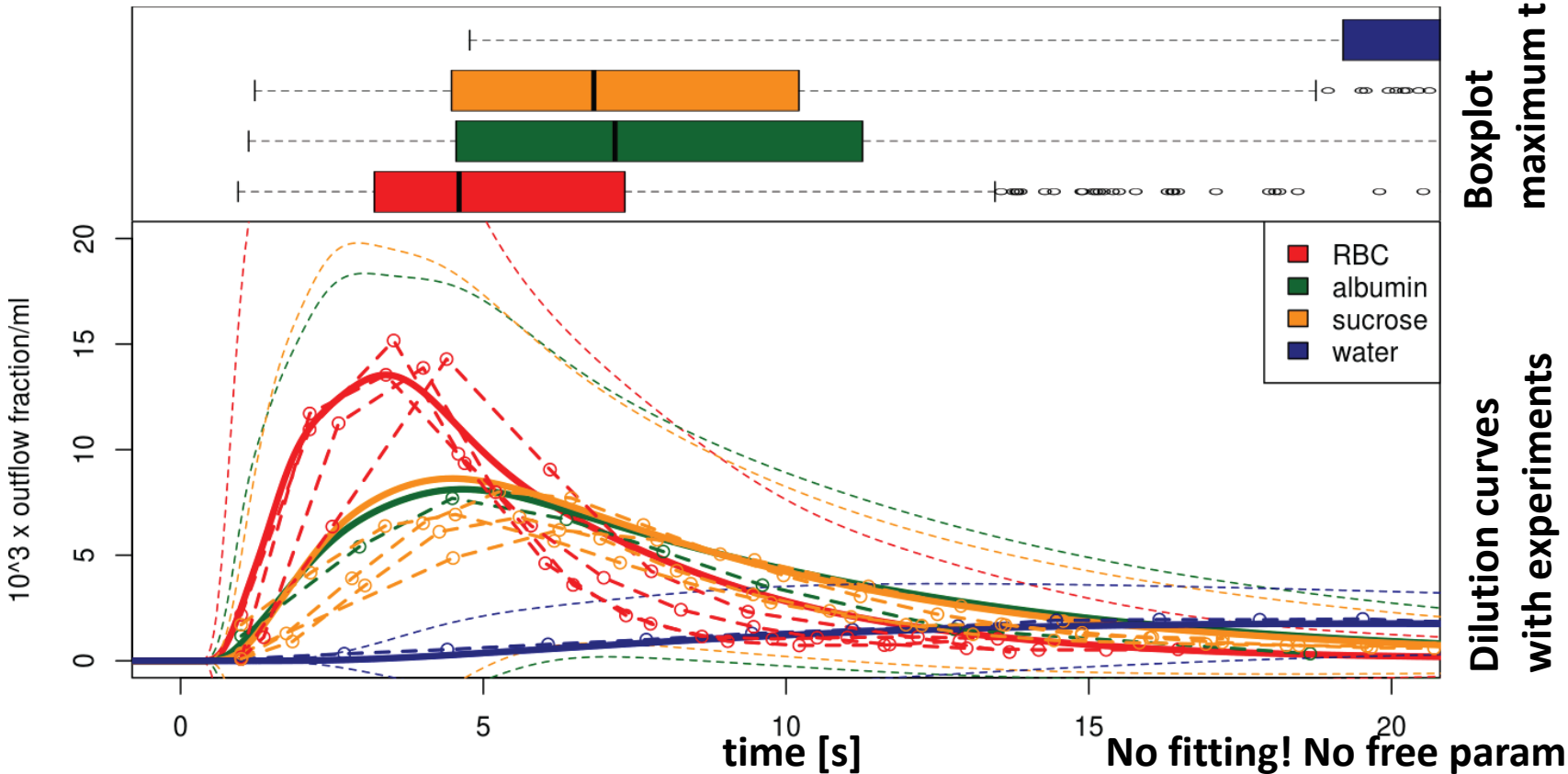
Periportal tracer







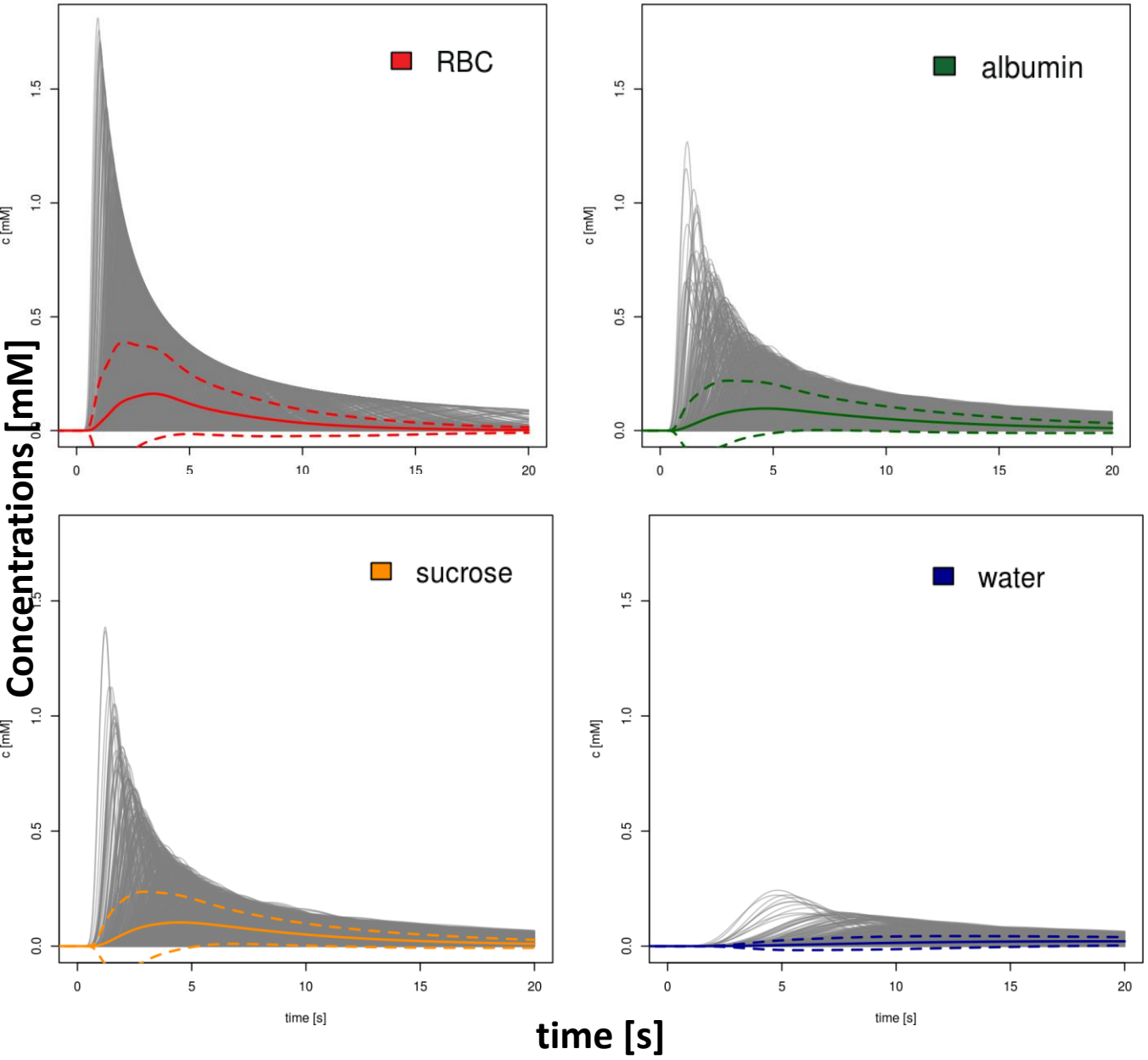
Perivenious tracer

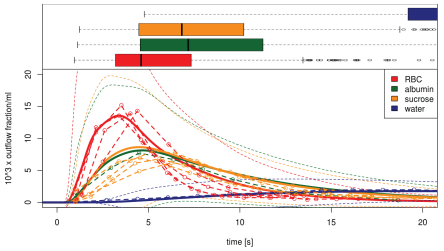


Boxplot maximum times

Dilution curves with experiments

No fitting! No free parameters!





- **Extreme heterogeneity** (!)
  - Local blood flow & tissue architecture
- **Strong effects on**
  - Drug clearance
  - Metabolic function
  - Signalling
  - …

GALACTOSE ELIMINATION (GE)

$$GE = (c_a^{gal} - c_v^{gal})$$

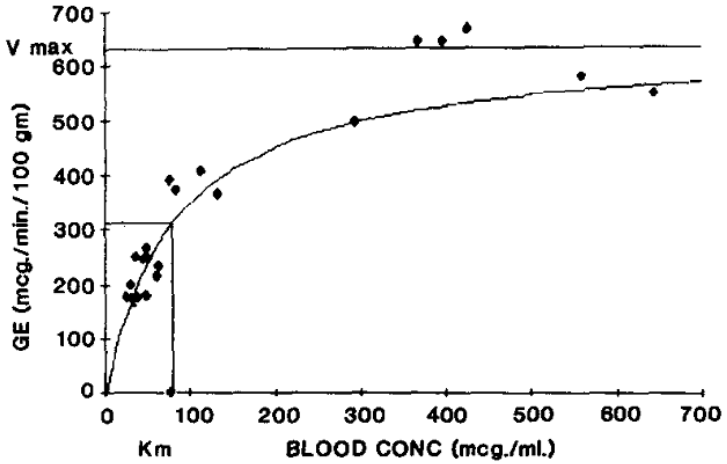
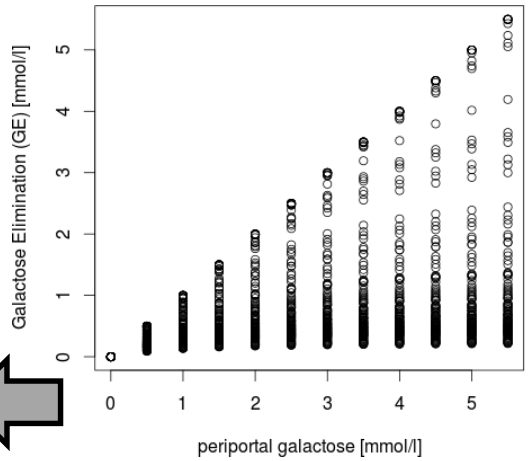
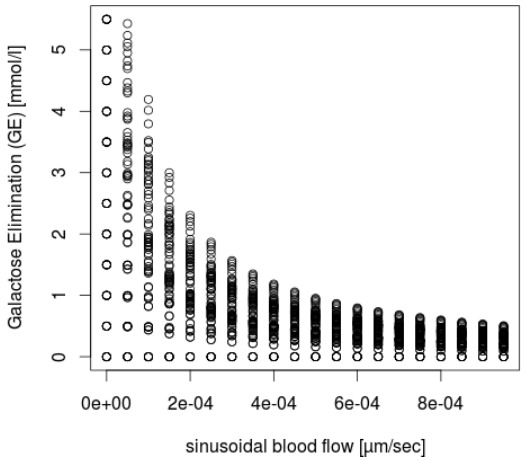
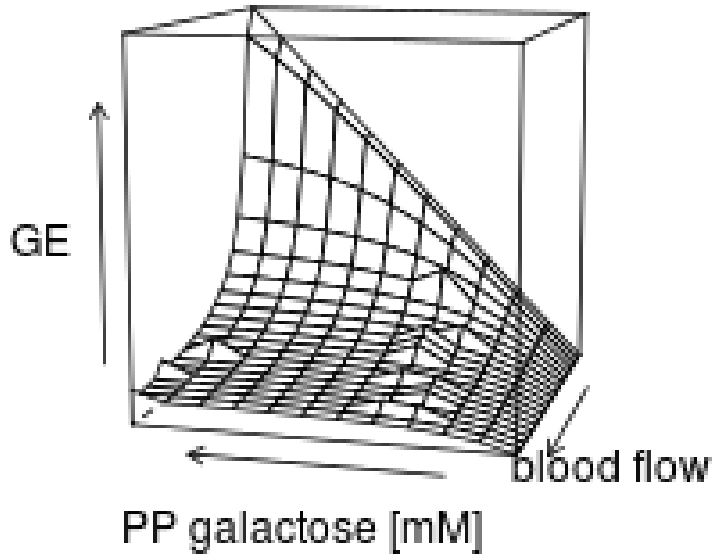


FIG. 1. Galactose elimination kinetics. Points represent individual animals. Superimposed line as determined by the Michaelis–Menten equation using the elimination constants, V_{max} and K_m, from Fig. 2.







EXTRACTION (ER) & CLEARANCE (CL)

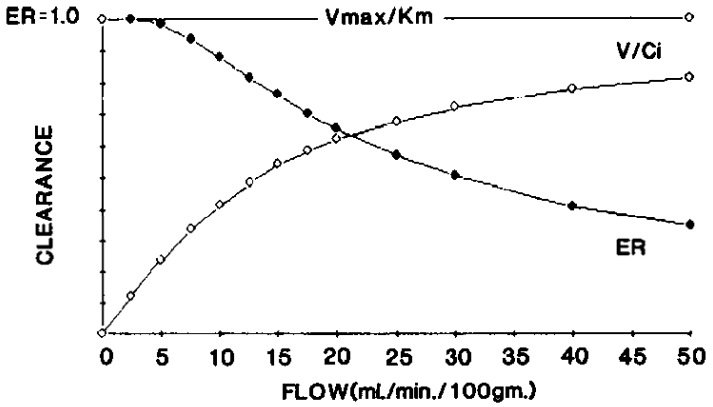
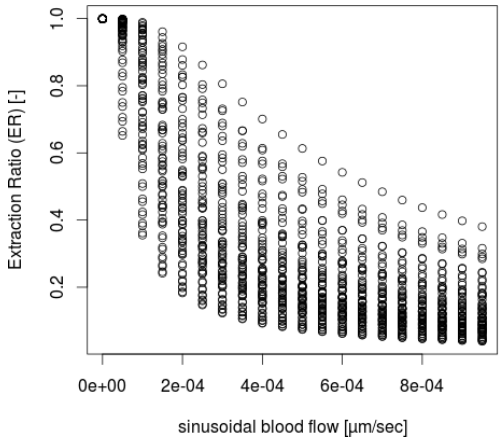
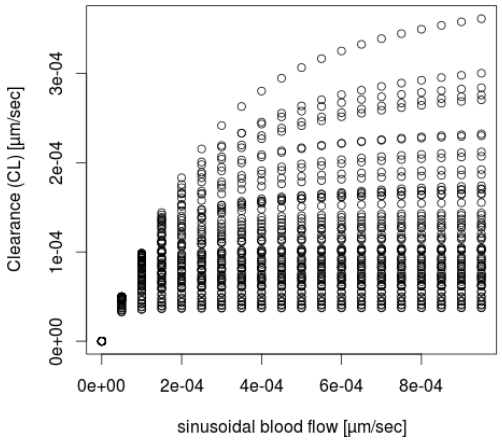


FIG. 6. Clearance and extraction ratio vs flow. Extraction ratio decreases as flow increases. Clearance increases with flow to a maximum of V_{max}/K_m.



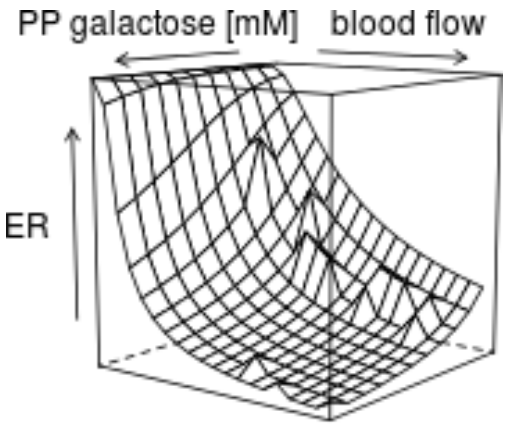


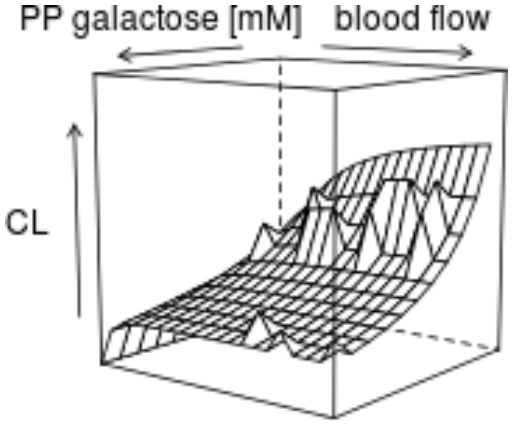
$$GE = (c_a^{gal} - c_v^{gal})$$

$$R = F \cdot (c_a^{gal} - c_v^{gal})$$

$$ER = \frac{(c_a^{gal} - c_v^{gal})}{c_a^{gal}}$$

$$Cl = \frac{R}{c_a^{gal}} = Q_{tot} \cdot \frac{(c_a^{gal} - c_v^{gal})}{c_a^{gal}}$$





CURRENT WORK

- Predict alterations
  - GEC in aging
  - Cirrhosis
- Analyse galactosemias
- Use spatially resolved PET data (individual subjects)
  - Normal
  - cirrhosis

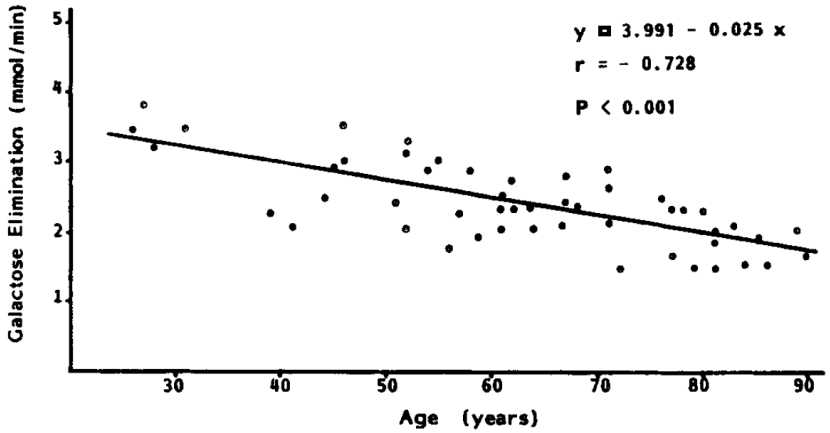


FIG. 1. Correlation between age and galactose elimination capacity in subjects of different age groups.

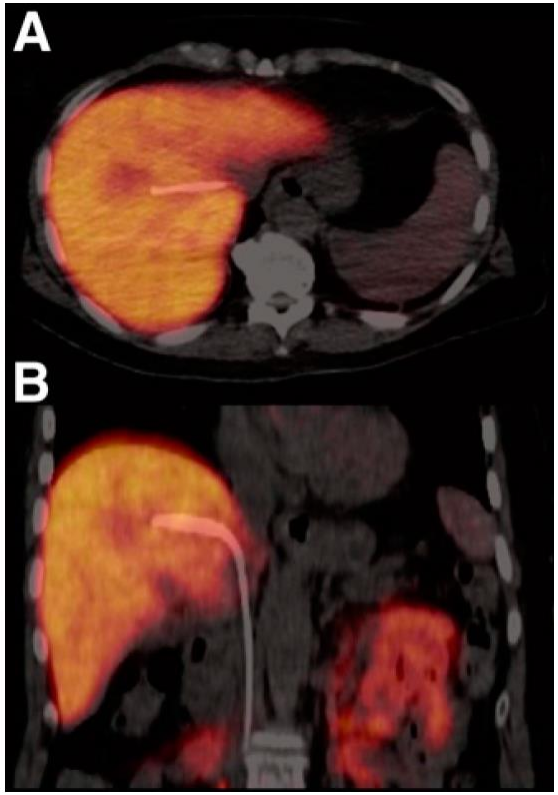


FIGURE 1. Transaxial (A) and coronal (B) ^{18}F-FDGal PET/CT images of mean tissue radioactivity concentration (kBq/mL liver tissue) from PET study without galactose infusion (subject 6). The position of the liver vein catheter inserted via the right femoral vein is seen. Note that the tracer accumulates specifically in liver tissue.

WHAT I LEARNED ... SO FAR

- Multiscale requires data management
  - **only reproducible results are results**
  - Database layer **decouples simulation & analysis**
  - Structured/standard formats function as **model unit tests**
- Multiscale relies on units
  - **a model without units is no model** (what is the meaning of parameters and componets?)
  - automatic unit checks as **model unit tests**
  - Units do the modelling for you (tell you how the system produces the quantity)
- Multiscale is difficult & more easy
  - Hierarchical constraints fix parameters
  - Every scale provides a new set of experimental data
- Multiscale-errors (!)
  - The more scales the more obscure the errors
  - Visualize everything

ACKNOWLEDGEMENTS

- **Concept & Modeling**
  - Prof. Holzhütter
- **Experimental Partners**
  - Prof. Tygstrup
  - Prof. Marchesini
- **SBML & SED-ML**
  - Frank Bergmann, Lucian Smith, Mike Hucka, Sarah Keating
- **SABIO-RK & data management**
  - Martin Golibiewski
  - Wolfgang Müller
  - Renate Wittig
- **Funding**
  - Charité
  - Virtual Liver Network

PROCESSES

- SBML Model

http://seek.virtuelle-leber.de/models/138

- Annotations
  - CHEBI, UniProt, KEGG, RHEA
  - kinetic parameters linked to SABIO-RK

Galactokinase

$$V_{\max}^{GALK} = S_f \cdot PA^{GALK} \cdot k_{cat}^{GALK}$$

$$v^{GALK} = \frac{V_{\max}^{GALK}}{k_{gal}^{GALK} k_{atp}^{GALK} \left(1+\frac{[gal1p]}{ki_{gal1p}^{GALK}}\right)} \cdot \frac{[gal][atp] - \frac{[gal1p][adp]}{k_{eq}^{GALK}}}{\left(1+\frac{[gal]}{k_{gal}^{GALK}}\right)\left(1+\frac{[atp]}{k_{atp}^{GALK}}\right) + \left(1+\frac{[gal1p]}{k_{gal1p}^{GALK}}\right)\left(1+\frac{[adp]}{k_{adp}^{GALK}}\right) - 1}$$

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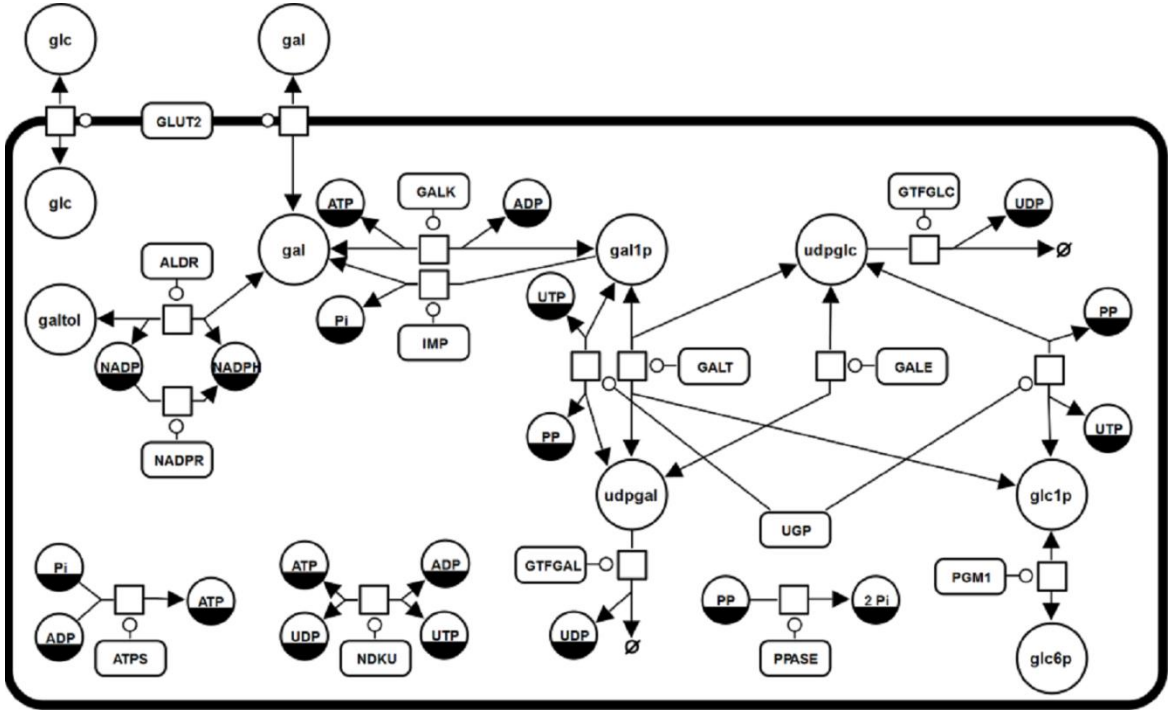
SABIO-RK Biochemical Reaction Kinetics Database

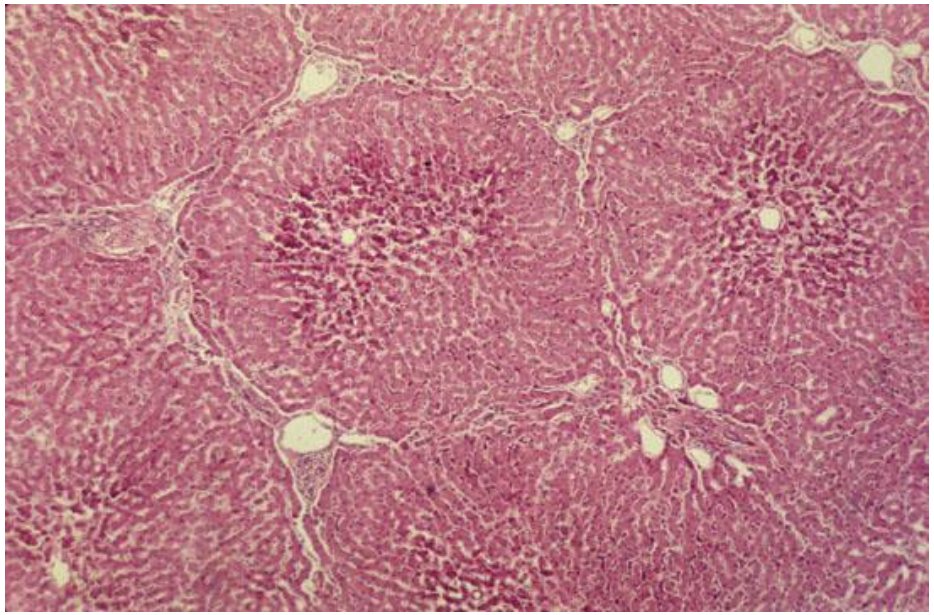
Kinetic data	Reaction	Enzyme			Tissue	Organism	Parameter (besides concentration)	Environment		Add to export cart?
		ECNumber	Protein	Variant				°C	pH	
▼	D-Galactose + ATP = ADP + alpha-D-Galactose 1-phosphate	2.7.1.6	P51570	wildtype	-	Homo sapiens	Kcat Kcat/Km Km	37.0	8.0	
Entry ID: 14785										

Parameter							
name	type	species	start val.	end val.	deviat.	unit	comment
E	concentration	Enzyme	32.0	67.0	-	nM	-
A	concentration	D-Galactose	0.0	2000.0	-	µM	-
kcat	kcat	-	8.7	-	5	s^(-1)	-
kcat_Km	kcat/Km	D-Galactose	8900.0	-	2900	M^(-1)*s^(-1)	-
Km	Km	D-Galactose	970.0	-	220	µM	-



Reactions: (ALDR) **Aldose reductase (galactitol NAD 1-oxidoreductase);** (ATPS) **ATP synthesis;** (GALDH) **Galactose 1-dehydrogenase; (**GALE) **UDP-glucose 4-epimerase;** (GALK) **Galactokinase;** (GALT) **Galactose-1-phosphate uridyl transferase;** (GLUT2) **Facilitated glucose transporter member 2;** (GTFGAL) **Glycosyltransferase galactose;** (GTFGLC) **Glycosyltransferase glucose;** (NADPR) **NADP reductase;** (NDKU) **Nucleoside diphosphokinase, ATP:UDP phosphotransferase;** (IMP) **Inositol monophosphatase;** (PGM1) **Phosphoglucomutase-1;** (PPASE) **Pyrophosphatase;** (UGALP) **UDP-galactose pyrophosphorylase; (**UGP) **UDP-glucose pyrophosphorylase;**

Metabolites: (adp) **ADP;** (atp) **ATP;** (gal) **D-galactose;** (gal1p) **D-galactose 1-phosphate;** (galnat) **D-galactonate;** (galtol) **D-galactitol;** (glc) **D-glucose;** (glc1p) **D-glucose 1-phosphate;** (glc6p) **D-glucose 6-phosphate;** (nadp) **NADP;** (nadph) **NADPH;** (pi) **phosphate;** (pp) **pyrophosphate;** (udp) **UDP;** (udpgal) **UDP-D-galactose;** (udpglc) **UDP-D-glucose;** (utp) **UTP;**



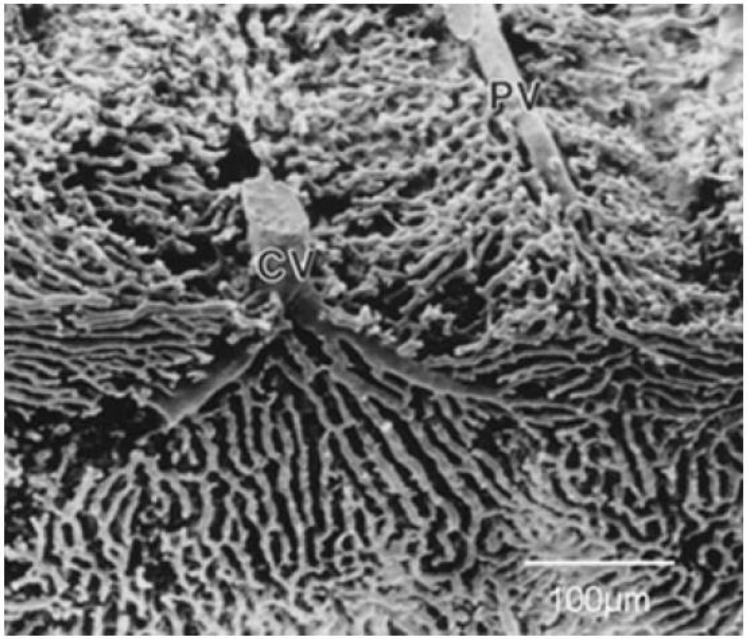


Fig. 2. Vascular cast of the hepatic microvasculature illustrating the tortuous, anastomotic sinusoids adjacent to the portal venule (PV) and the more parallel and larger sinusoids near the central venule (CV) (McCuskey, 1993).

SINUSOID

- principal vessels for exchange between blood and hepatocytes
- ~ 6-8µm diameter
- periportal sinusoids are narrower and more tortuous than the wider and straighter central ones
- Sinusoid network is heterogeneous
  - **near portal vene** arranged as **interconnecting polygonal networks**
  - farther away from portal vein organized as **parallel vessels terminating in the central vein**
  - short **intersinusoidal sinusoids** connect adjacent parallel sinusoids

Scanning electron micrograph showing fenestrated sinusoids and hepatocytes in a mouse liver.
http://www.easloffice.eu/jhep/contest/website/see_photos.html

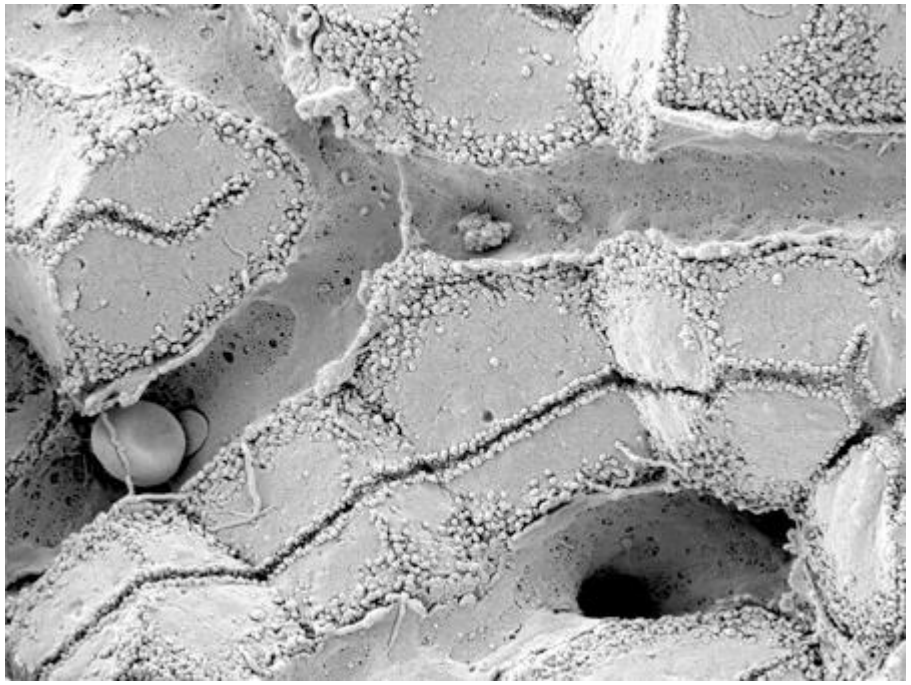


TABLE 1. COMPARISON OF MEASUREMENTS ON SINUSOIDS AND BLOOD CELLS IN MICRONS ± S.E.

	In vivo/LM[a]	In plastic/LM	After CPD/SEM
Portal sinusoid	5.9 ± 0.17 (n = 545, 6 rats)	6.42 ± 0.12 (n = 696, 2 rats)	4.09 ± 0.06 (n = 1, 452, 10 rats)
Central sinusoid	7.1 ± 0.29 (n = 498, 6 rats)	7.62 ± ND[b] (n = 696, 2 rats)	5.67 ± ND (n = 1, 452, 10 rats)

Scanning electron microscope observations on the structure of portal veins, sinusoids and central veins in rat liver. Wisse, E.; De Zanger, R. B.; Jacobs, R. & McCuskey, R. S.; *Scan Electron Microsc,* **1983**, 1441-1452
The liver sieve: considerations concerning the structure and function of endothelial fenestrae, the sinusoidal wall and the space of Disse. Wisse, E.; De Zanger, R. B.; Charels, K.; Van Der Smissen, P. & McCuskey, R. S. *Hepatology,* **1985**, *5*, 683-692

BLOOD FLOW

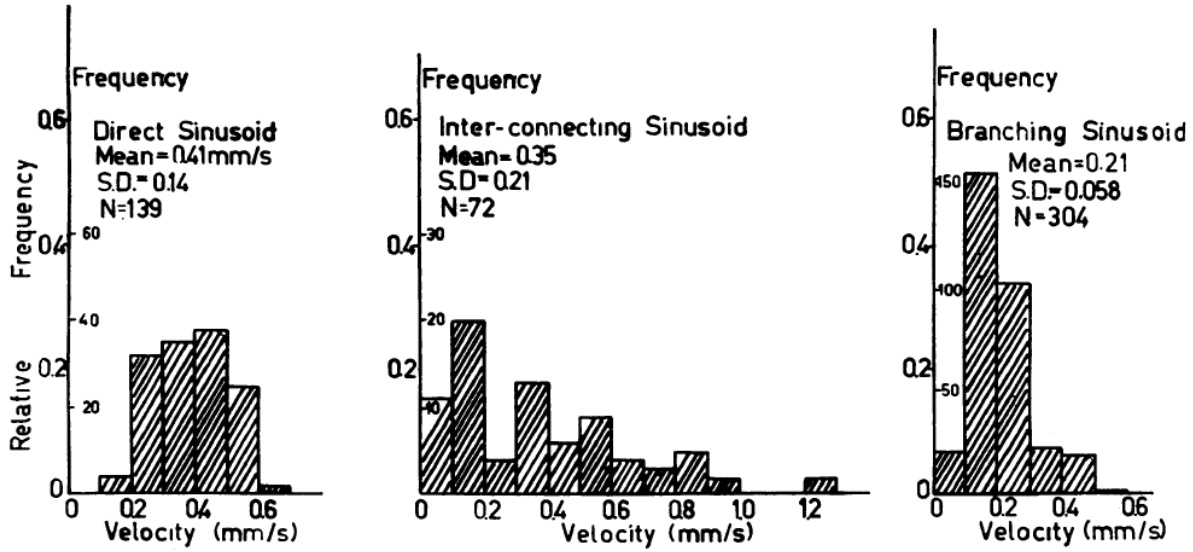
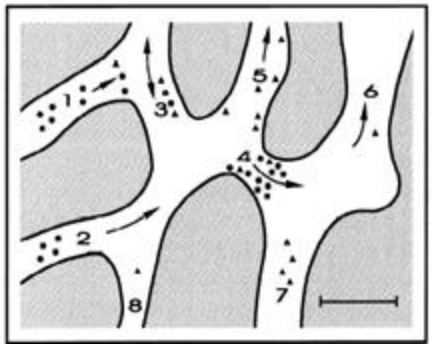


FIG. 1. Frequency distribution of the velocity of the erythrocytes in the direct sinusoids, the branching sinusoids and the interconnecting sinusoids.



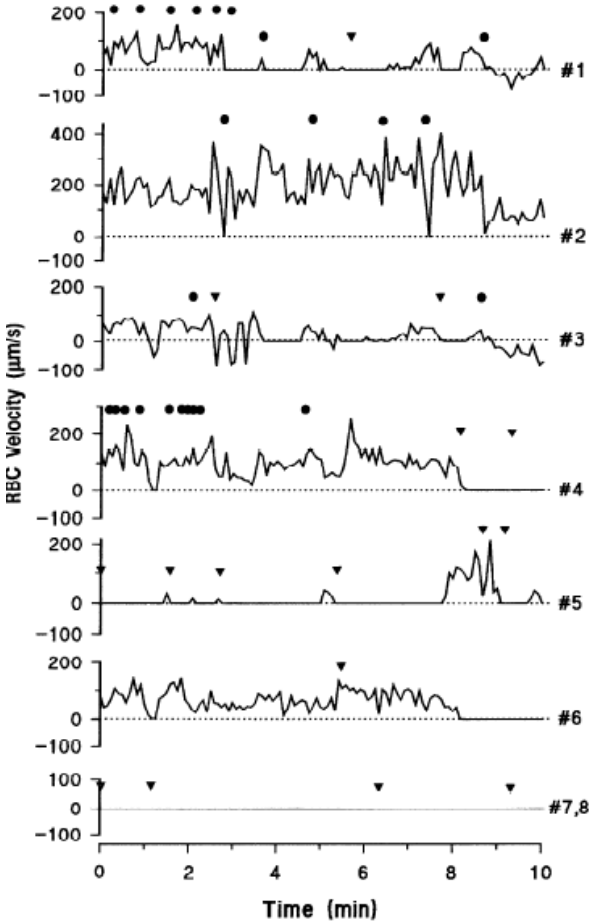


Fig. 3. Temporal overview of RBC velocity fluctuations over a 10-min period, in sinusoidal network from zone 1 of mouse liver (shown in Fig. 2). Measurements in each sinusoid were made every 5 s. Changes of flow in one sinusoid often produced changes in others. Instants are indicated at which a migrating Kupffer cell obstructed flow (▼) or a circulating leukocyte slowed or stopped temporarily (●). *Sinusoids 7* and *8* had no flow throughout the 10-min period. Kupffer cells are seen in *sinusoid 7*, and in *sinusoid 8* one Kupffer cell blocked flow throughout.

The terminal hepatic microcirculation in the rat.
Koo, A.; Liang, I. Y. & Cheng, K. K.; *Q J Exp Physiol Cogn Med Sci,* **1975**, *60*, 261-266
Intermittence of blood flow in liver sinusoids, studied by high-resolution in vivo microscopy. MacPhee, P. J.; Schmidt, E. E. & Groom, A. C.; **1995**, *269*, G692-G698

GALACTOSEMIAS

- caused by **deficiencies in either GALK, GALT or GALE**
- untreated as well as treated patients with galactosemia show accumulation and/or depletion of specific metabolites, and often abnormalities of glycosylation

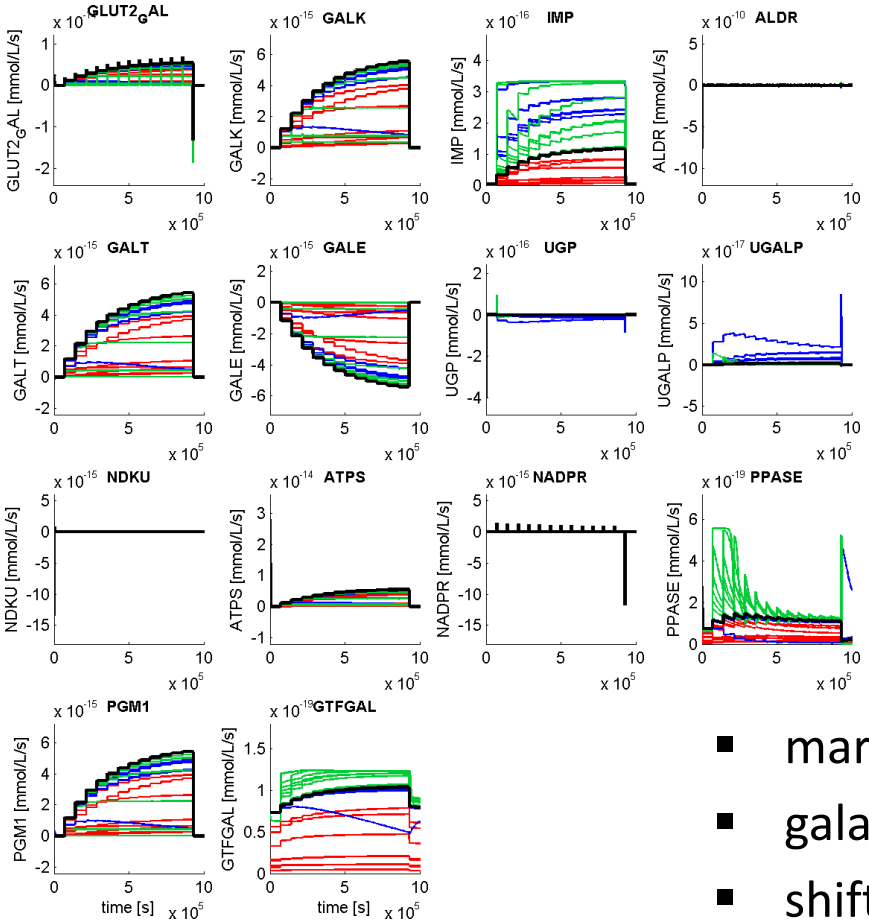
implementation

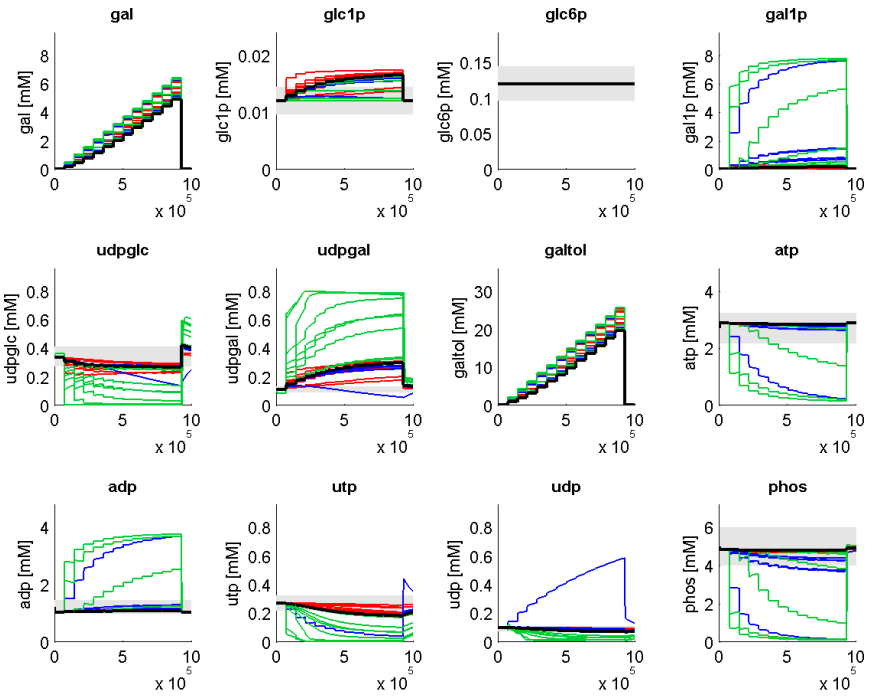
- via measured alterations in kinetic properties in human protein mutations

Table 4 *- Kinetic parameters in GALK, GALT and GALE deficiencies.*

	Enzyme	Variant	k_{cat} [1/s] (%wt)	K_m(gal) [mM] (%wt)	K_m(atp) [mM] (%wt)	Reference
	GALK	Wild Type	8.7±0.5 (100)	0.97±0.22 (100)	0.034±0.004 (100)	[51]
1	GALK	H44Y	2.0±0.1 (23)	7.70±4.40 (794)	0.130±0.009 (382)	[51]
2	GALK	R68C	3.9±0.8 (45)	0.43±0.15 (44)	0.110±0.035 (324)	[51]
3	GALK	A198V	5.9±0.1 (68)	0.66±0.22 (68)	0.026±0.001 (76)	[51]
4	GALK	G346S	0.4±0.04 (5)	1.10±0.16 (113)	0.005±0.002 (15)	[51]
5	GALK	G347S	1.1±0.2 (13)	13.0±2.0 (1340)	0.089±0.034 (262)	[51]
6	GALK	G349S	1.8±0.1 (21)	1.70±0.48 (175)	0.039±0.004 (115)	[51]
7	GALK	E43A	6.7±0.02 (77)	1.90±0.50 (196)	0.035±0.0003 (103)	[100]
8	GALK	E43G	0.9±0.02 (10)	0.14±0.01 (14)	0.0039±0.0006 (11)	[100]
	Enzyme	**Variant**	**V_{max} [nmol/mg/s] (% wt)**	**K_m(gal1p) [mM] (%wt)**	**K_m(udpglc) [mM] (%wt)**	**Reference**
	GALT	Wild Type	804±65 (100)	1.25±0.36 (100)	0.43±0.09 (100)	[22]
9	GALT	R201C	396±59 (49)	1.89±0.62 (151)	0.58±0.13 (135)	[22]
10	GALT	E220K	253±53 (31)	2.34±0.42 (187)	0.69±0.16 (160)	[22]
11	GALT	R223S	297±25 (37)	1.12±0.31 (90)	0.76±0.09 (177)	[22]
12	GALT	I278N	45±3 (6)	1.98±0.35 (158)	1.23±0.28 (286)	[22]
13	GALT	L289F	306±23 (38)	2.14±0.21 (171)	0.48±0.13 (112)	[22]
14	GALT	E291V	385±18 (48)	2.68±0.16 (214)	0.95±0.43 (221)	[22]
	Enzyme	**Variant**	**k_{cat} [1/s] (%wt)**	**K_m(udpglc) [mM] (%wt)**		**Reference**
	GALE	Wild Type	36±1.4 (100)	0.069±0.012 (100)		[59]
15	GALE	N34S	32±1.3 (89)	0.082±0.015 (119)		[59]
16	GALE	G90E	0.046±0.0028 (0)	0.093±0.024 (135)		[59]
17	GALE	V94M	1.1±0.088 (3)	0.160±0.038 (232)		[59]
18	GALE	D103G	5.0±0.23 (14)	0.140±0.021 (203)		[59]
19	GALE	L183P	11±1.2 (31)	0.097±0.040 (141)		[59]
20	GALE	K257R	5.1±0.29 (14)	0.066±0.015 (96)		[59]
21	GALE	L313M	5.8±0.36 (16)	0.035±0.011 (51)		[59]
22	GALE	G319E	30±1.3 (83)	0.078±0.013 (113)		[59]
23	GALE	R335H	15±0.48 (42)	0.099±0.012 (143)		[59]

GALACTOSEMIA (GALK, GALT, GALE)





- marked alterations in metabolite levels
- galactitol accumulation
- shift of udpglc/udpgal level
- reduced galactose clearance
- energetically challenged