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Interplay between enzymes and transporters in defining hepatic drug clearance and intracellular concentration of drugs

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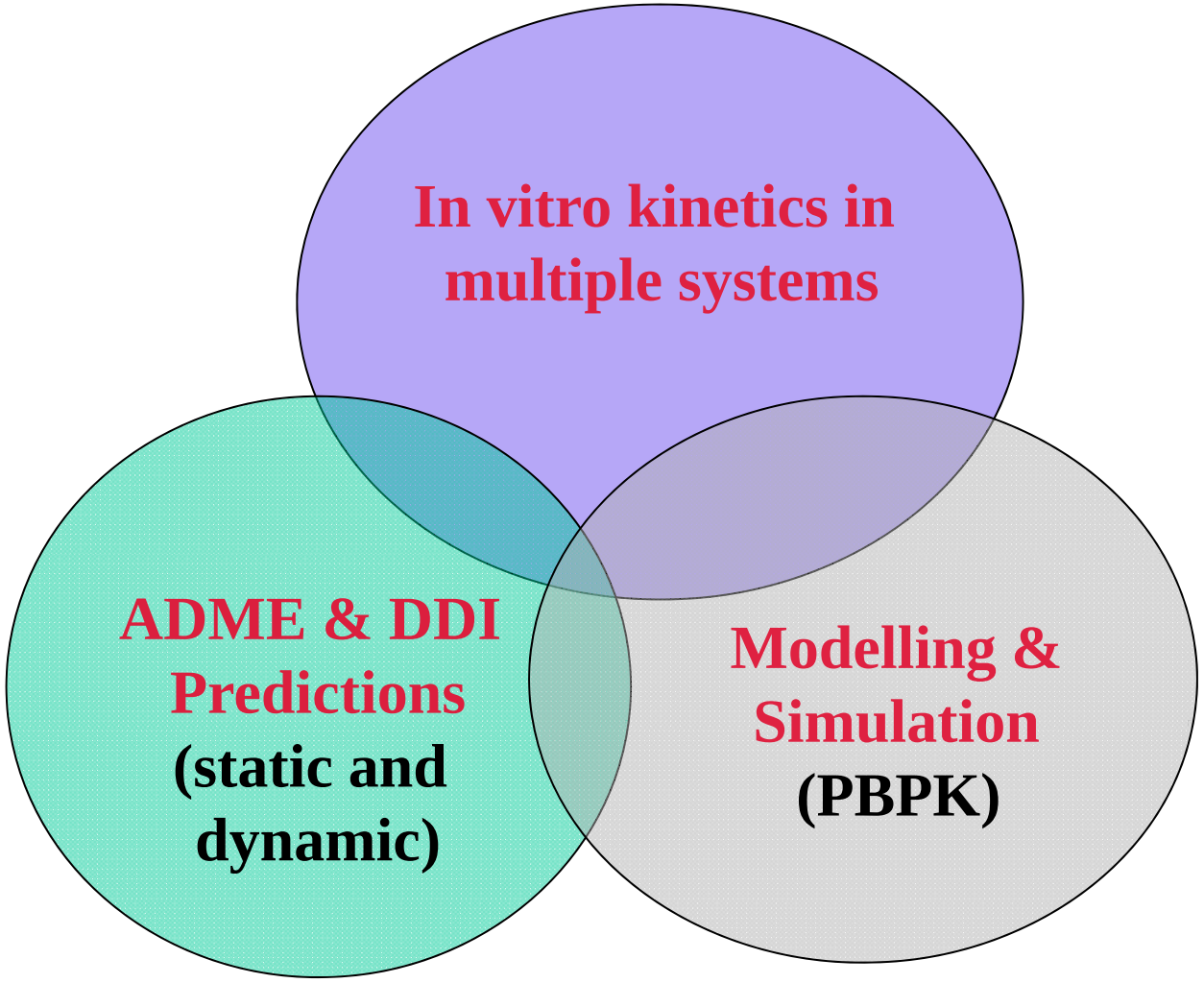
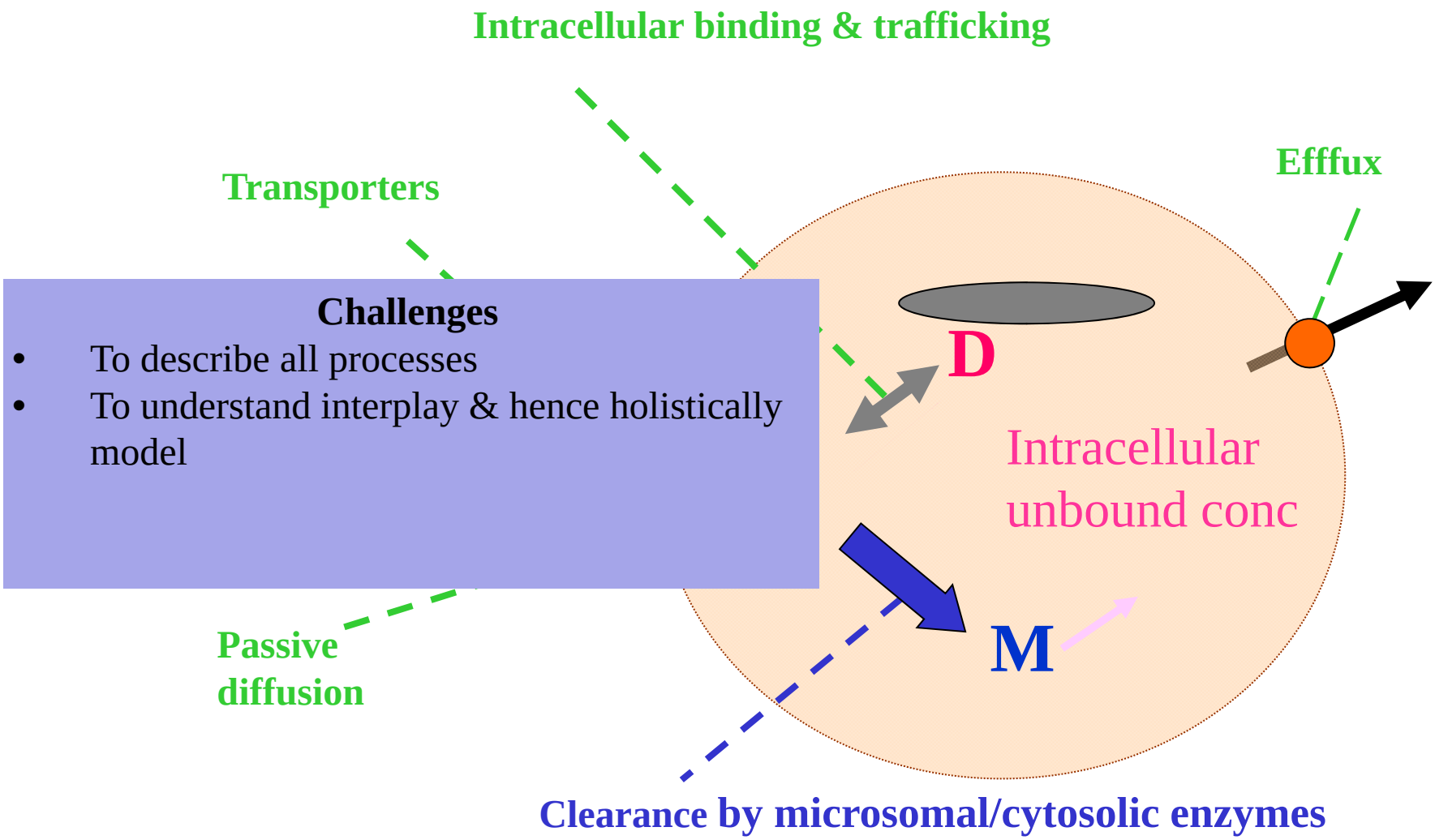
Three elements of mechanism-based prediction of human PK



Generic view of drug kinetics in hepatocytes:

Various processes defining drug intracellular concentration



Extending Classic Hepatic Clearance Models:

Use of $CL_{int,app}$ to delineate transporters & enzymes and their 'Interplay'

Prediction (static) equations well established:

e.g. well-stirred liver model

$$CL = \frac{Q_h \cdot fu_b \cdot CL_{int}}{Q_h + fu_b \cdot CL_{int}}$$

Extended with use of $Cl_{int,app}$ to encompass hepatocellular sequential processes (Interplay model) based on Sugiyama and Pang.

$$CL_{int,app} = CL_{int,met} \frac{CL_{int,active} + CL_{int,pass}}{CL_{int,met} + CL_{int,pass} + CL_{int,eff}}$$

$CL_{int,app}$ – Interplay of transporters and enzymes

$$CL_{Int,app} = CL_{Int,met} \frac{CL_{Int,active} + CL_{Int,pass}}{CL_{Int,met} + CL_{Int,pass} + CL_{Int,eff}}$$

- High passive permeability
  - ➢ reduces to $CL_{int,met}$
- Low passive permeability (with minimal effux)
  - ➢ reduces to $CL_{int,active}$
- Various intermediate cases. The second term collective – Kp_u (partition coefficient for unbound drug)

In vitro tools for assessment of hepatic uptake

- Hepatocytes:
  - Suspension culture - direct cell uptake (oil separation)
  - Plated cells (also sandwich configuration)
  - Single time points or full time course of uptake & metabolism
- Comparative scaled activity in hepatocytes relative to microsomes (subcellular preparation)
- Sometimes rat better option than human
  - Higher activity and less confounding issues surrounding preparation and storage
  - Minimal inter-individual variation
  - Potential extrapolation to human
- *CL* terms main metric, and for inhibition DDIs K_i

where $CL_i = CL_{control} / 1+K_i$

Characterisation of extent of hepatic uptake

What are we measuring with Kp_u?

- Kp_u = Cell to medium (plasma) unbound concentration ratio

$$Kp_u = \frac{CL_{\text{int},uptake} + CL_{\text{int},passive}}{CL_{\text{int},passive}}$$

[True Kpu - at steady state when no metabolism or efflux]

$$Kp_u = \frac{CL_{\text{int},pass} + CL_{\text{int},uptake}}{CL_{\text{int},pass} + CL_{\text{int},efflux} + CL_{\text{int},met}}$$

[Apparent Kp_u]

- Contrasts with Kp_{total} (ratio of total concentrations)

which reflects both uptake and intracellular binding

$$Kp_u = fu_{cell} \cdot Kp_{total}$$

Used together estimates intracellular drug concentration

Comparison of microsomes and hepatocytes

Evidence for hepatic uptake affecting CL and DDI prediction

If active uptake process occurring, substrate or inhibitor may show higher 'affinity' in hepatocytes compared to microsomes –

i.e. lower K_m or K_i as

$[S]_{u,plasma} << [S]_{u,liver}$ or $[I]_{u,plasma} << [I]_{u,liver}$

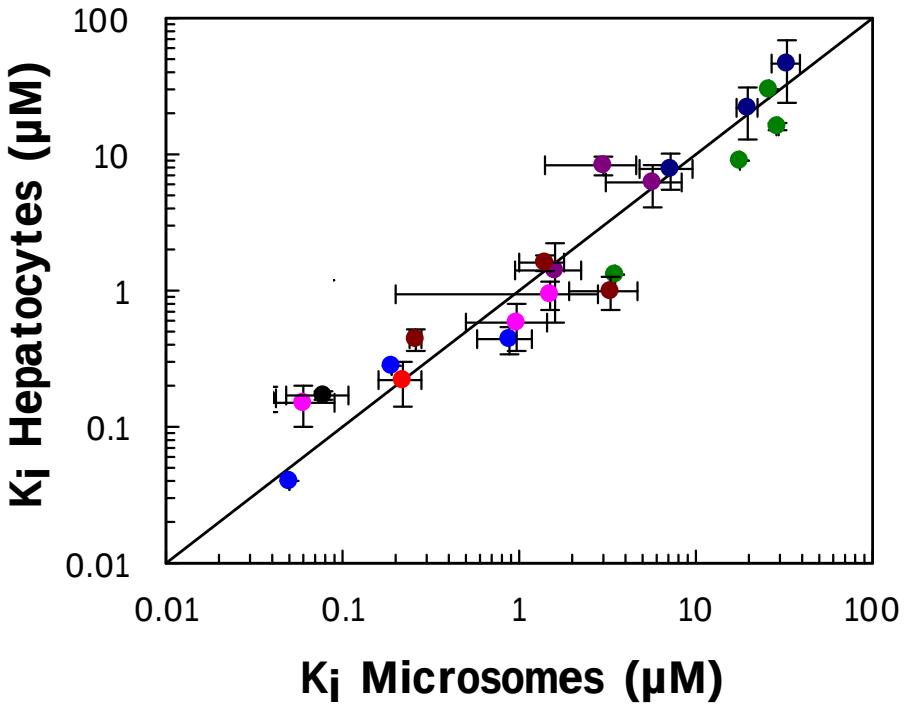
$$K_{p,u} = \frac{K_{i,microsome}}{K_{i,hepatocyte}}$$

$$= \frac{K_{m,microsome}}{K_{m,hepatocyte}} \qquad = \frac{CL_{int,hepatocyte}}{CL_{int,microsome}}$$

Impact of hepatic intracellular binding?:

K_i Microsomes vs. Hepatocytes

(n= 7 inhibitors, n=21 pathways)



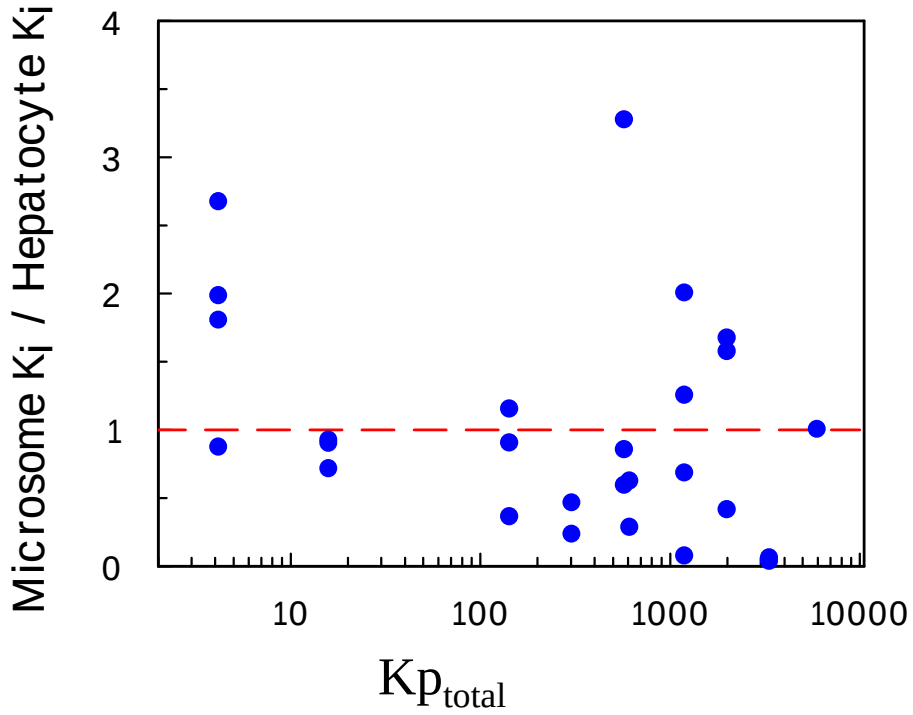
Inhibitor	Cell-to-Media Ratio (Kp_{total})
MCZ	6000
FXT	2010
KCZ	1200
FVX	577
QUI	143
OMP	16
FCZ	4.2

- Good agreement between K_i values in both systems (both corrected for non specific binding)
- Kp_u approximately 1

Brown et al, DMD 2007

Lack of impact of hepatic uptake:

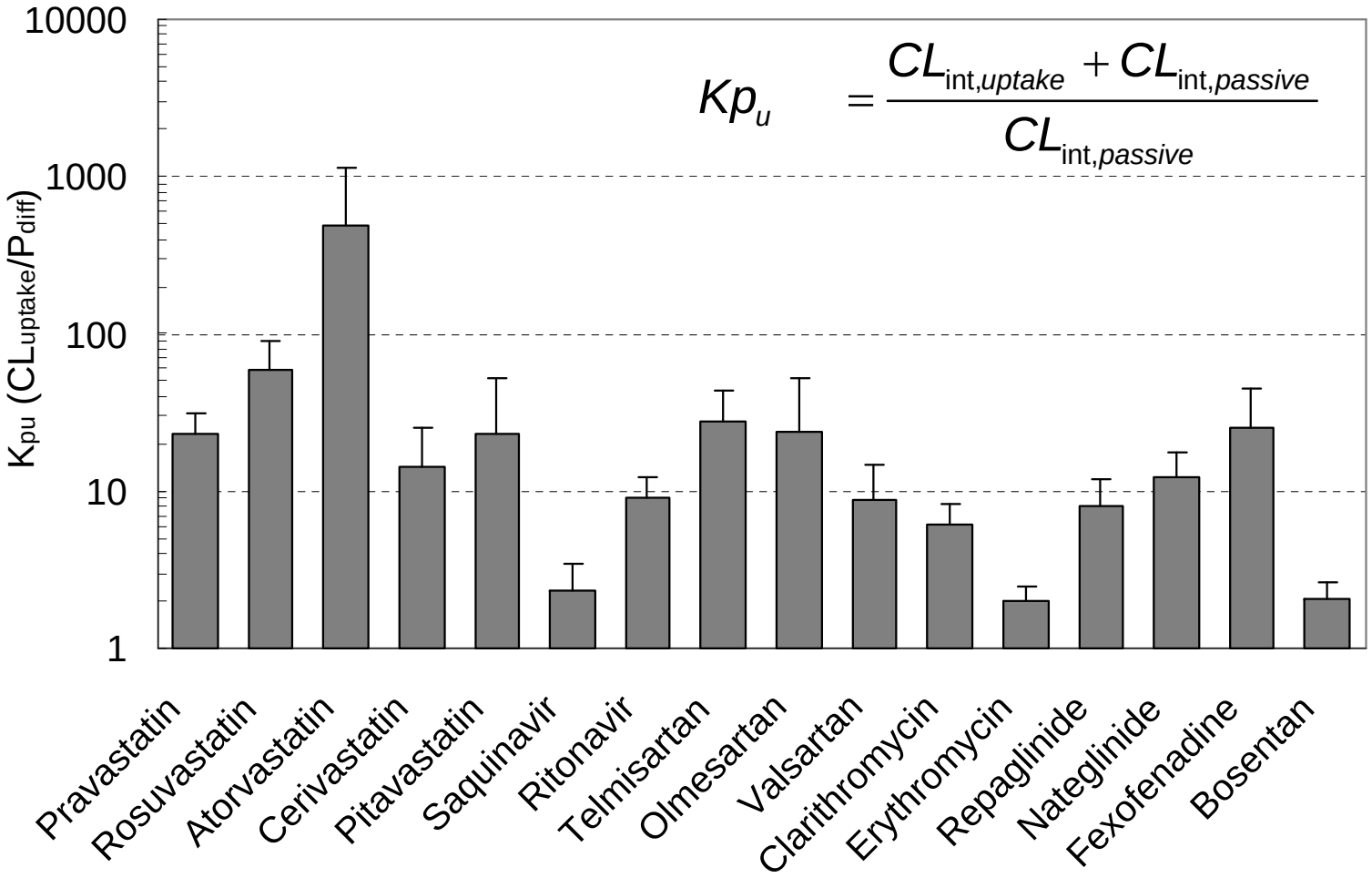
Microsomal & Hepatocyte K_i ratio vs. Kp_{total}



- Kp_{total} differ over 3 orders of magnitude
- No correlation between K_i ratio and Kp_{total} (n = 27)

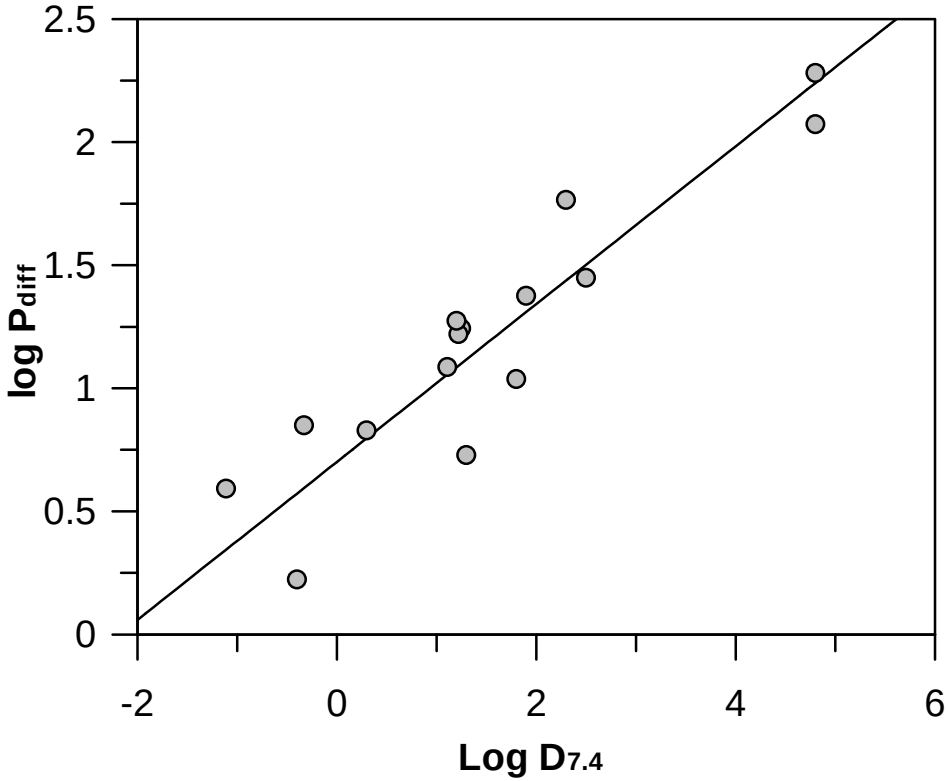
Brown et al, DMD 2007

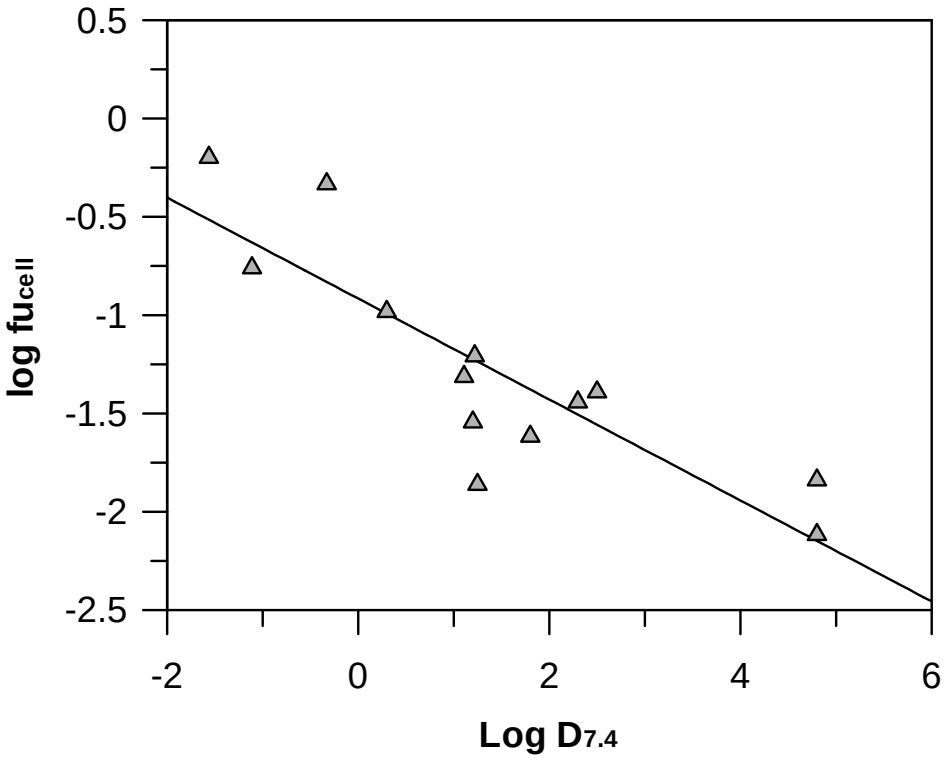
$Kp_u \neq 1$ – importance of uptake transporters for 16 drugs in rat (*Yabe et al DMD 2011*)



- **Kp_u (CL_{uptake}/P_{diff}) 250-fold range (erythromycin and atorvastatin).**

Covariate analysis





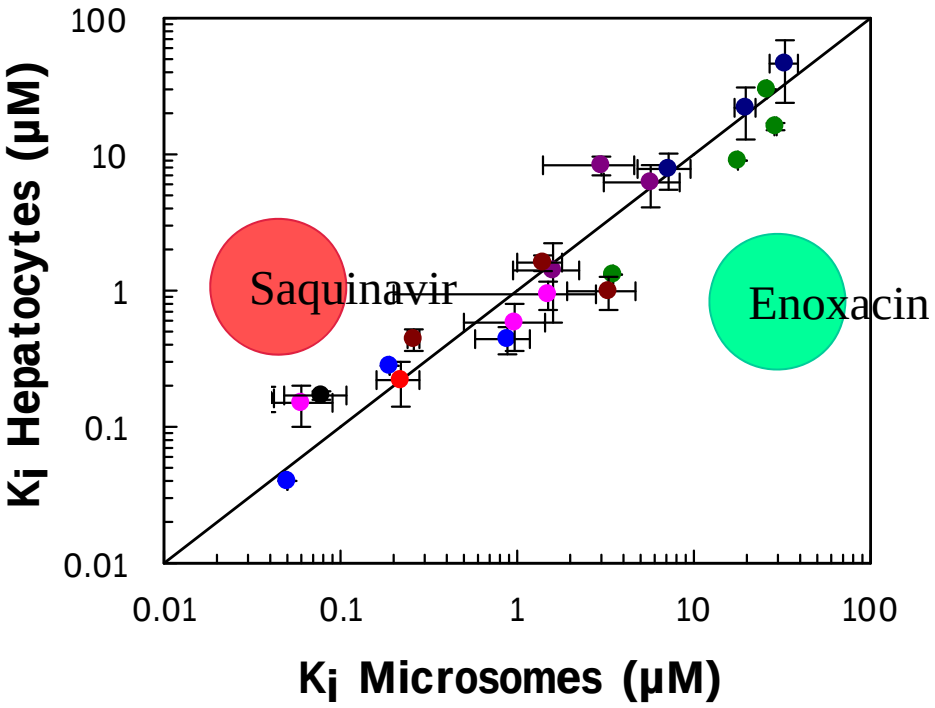
Useful for cross-species and cross-systems extrapolation

But no statistical relationship between:

Log D and any active uptake parameters

Interplay examples - $Kp_u \neq 1$: actively transported drugs

(n= 7 inhibitors, n=21 pathways)



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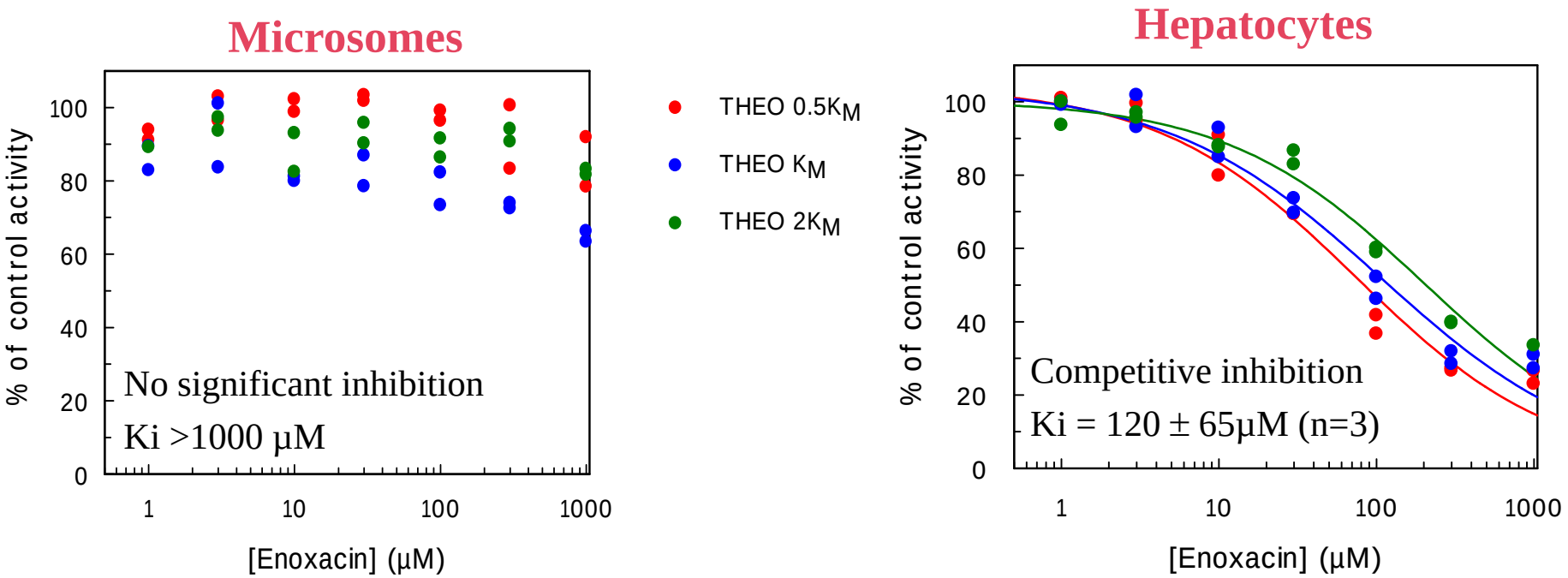
- Good agreement between K_i values in both systems (except the higher affinity inhibitors)
- Kpu approximately 1

Brown et al, DMD 2007

Example 1:Enoxacin inhibition of theophylline oxidation

Substantial DDI reported in humans and rats



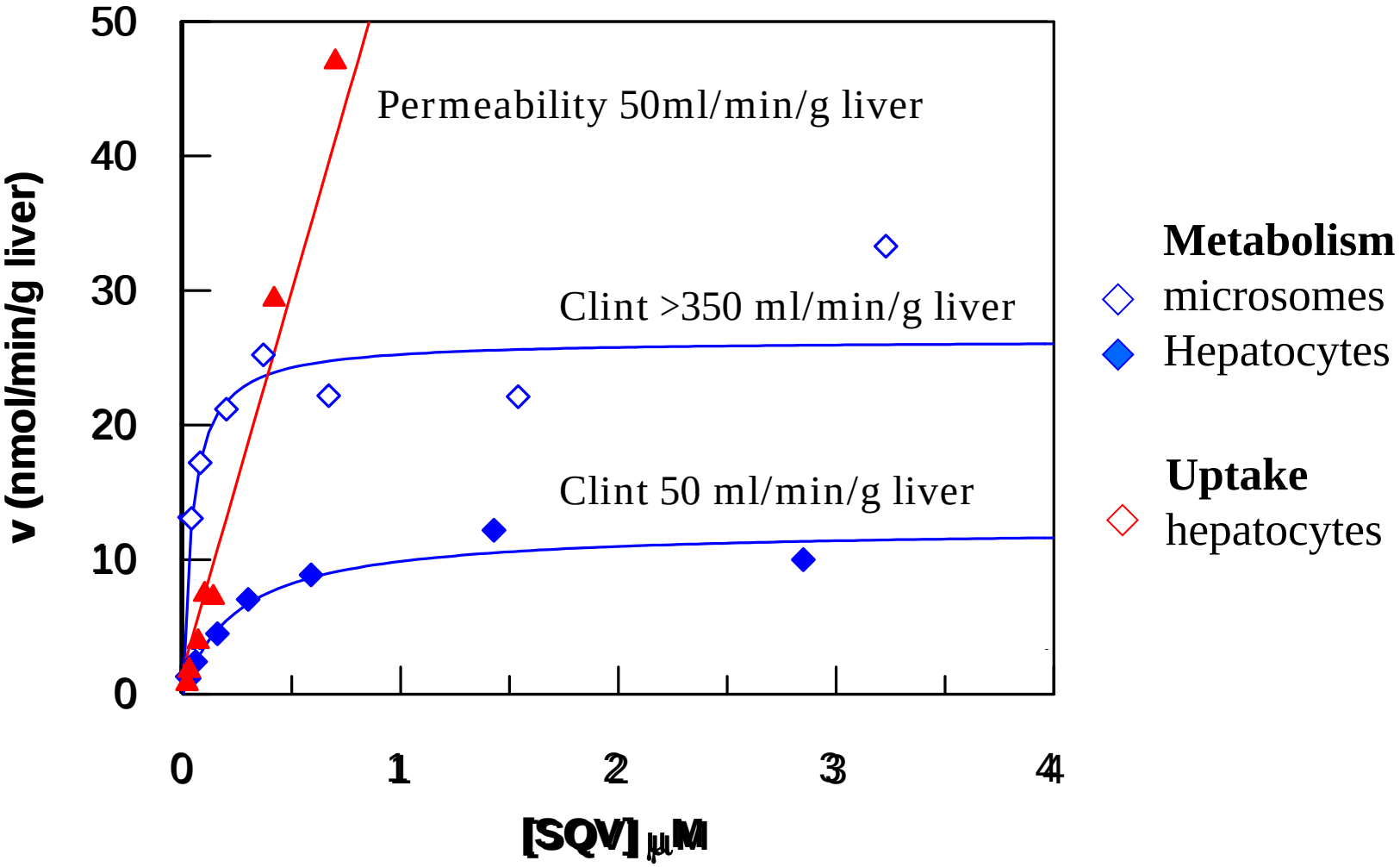
- Significantly more potent (>20) inhibition in cells vs. microsomes
- Active uptake of enoxacin
- Similar scenario observed with erythromycin

Brown et al, 2010

Example 2: Inhibition of CYP3A by HIV protease inhibitors – nelfinavir and saquinavir

- Well documented examples of actively transported drugs with substantial DDIs
- Hepatocyte-microsomal difference in Ki to be expected
- Similar scenario to enoxacin and erythromycin?

Saquinavir metabolism and uptake in rat



Uptake is rate limiting and defines CL

Hepatic uptake and microsomal:hepatocyte Km & Ki ratios for saquinavir and nelfinavir

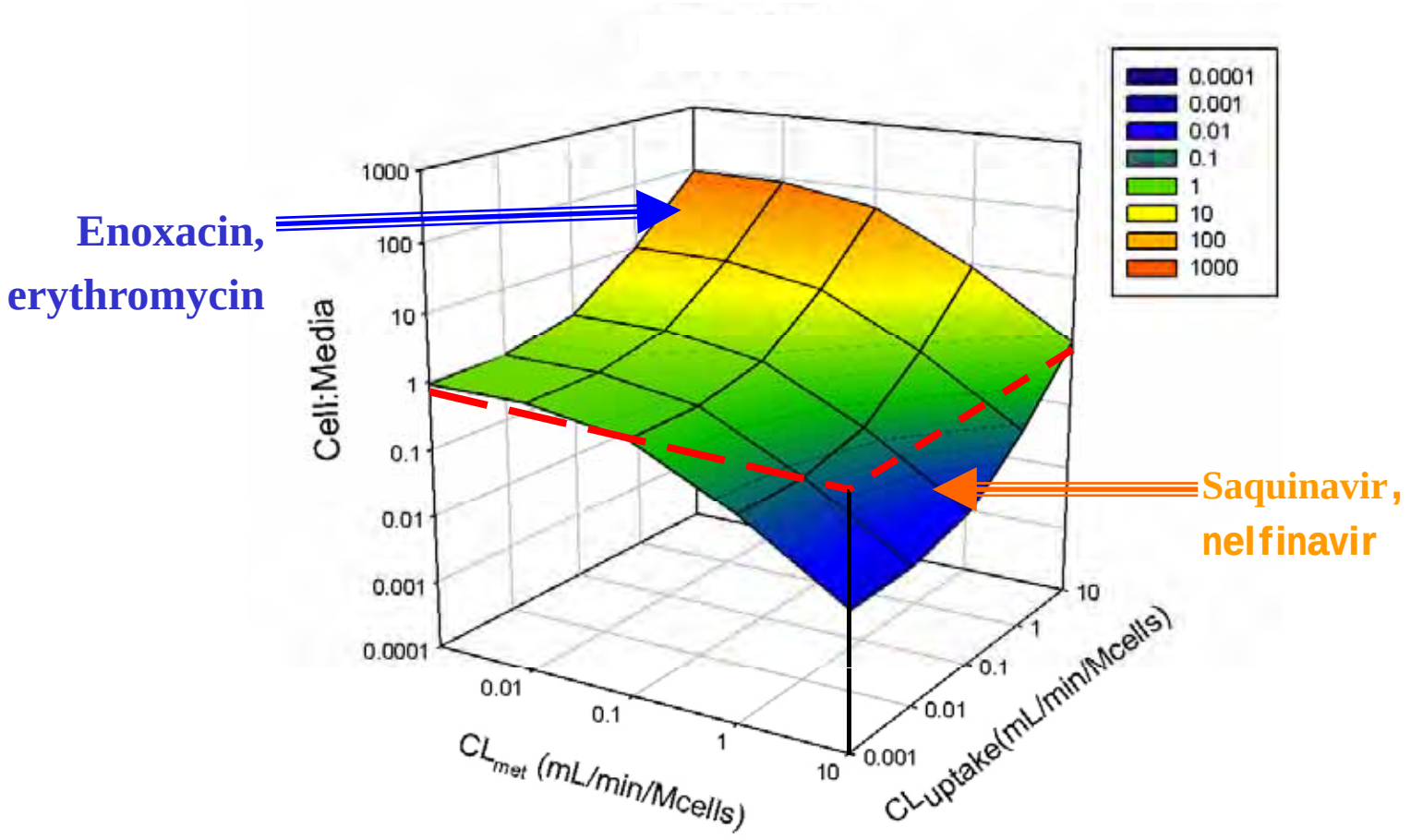
	Microsomal:hepatocellular ratio		
Drug (Kp_{total})	**K_m**	**K_i**	**K_{pu}**
Saquinavir (306)	0.16	0.34	6.8
Nelfinavir (3350)	0.03	0.04	5.7

Opposite effect to enoxacin & erythromycin cases

Parker et al, DMD 2008

Framework for interplay of metabolism & transporters on Kp_u

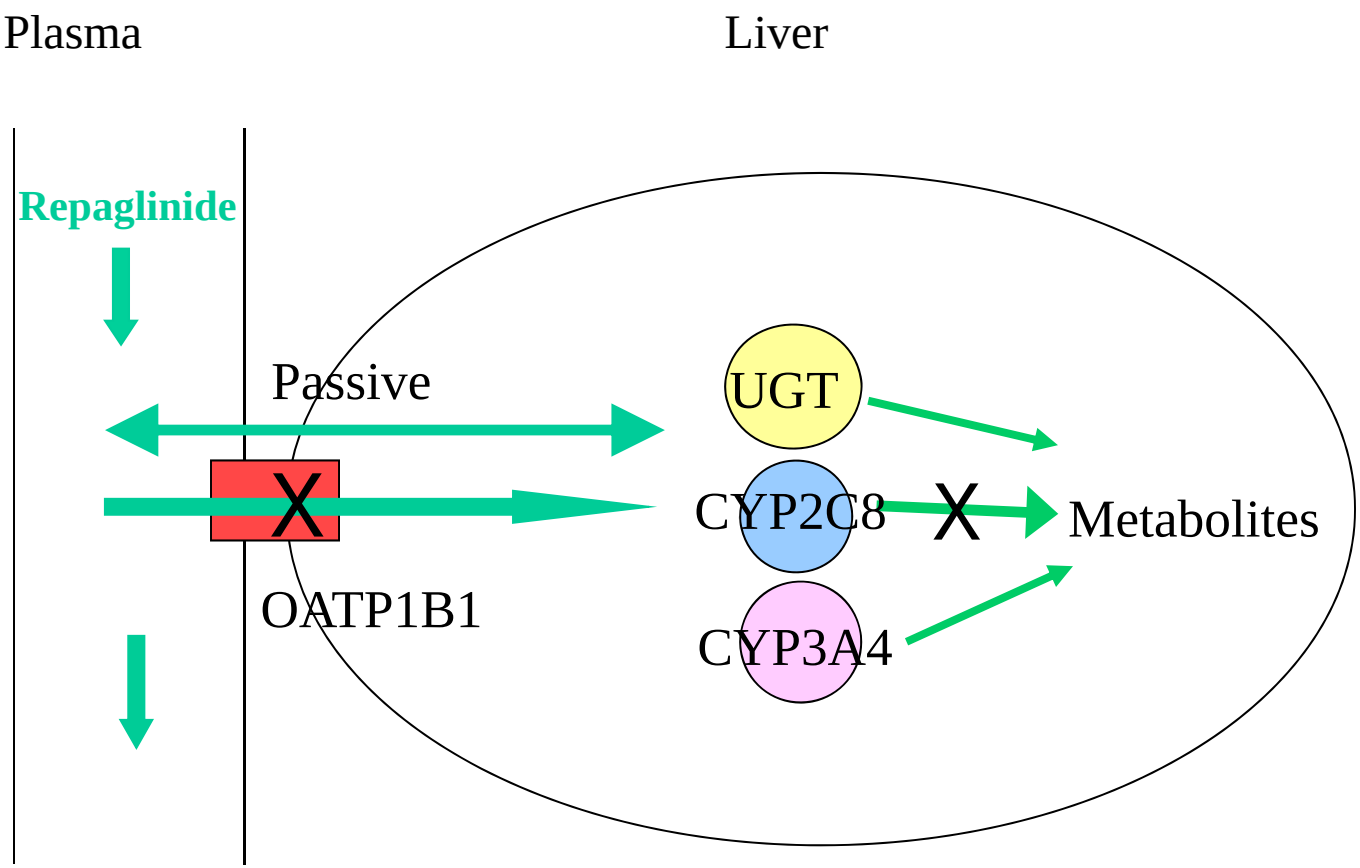
(Intermediate permeability 0.1 ml/min/M cells)



- No efflux or tissue binding

Example 3: Repaglinide-Gemfibrozil DDI

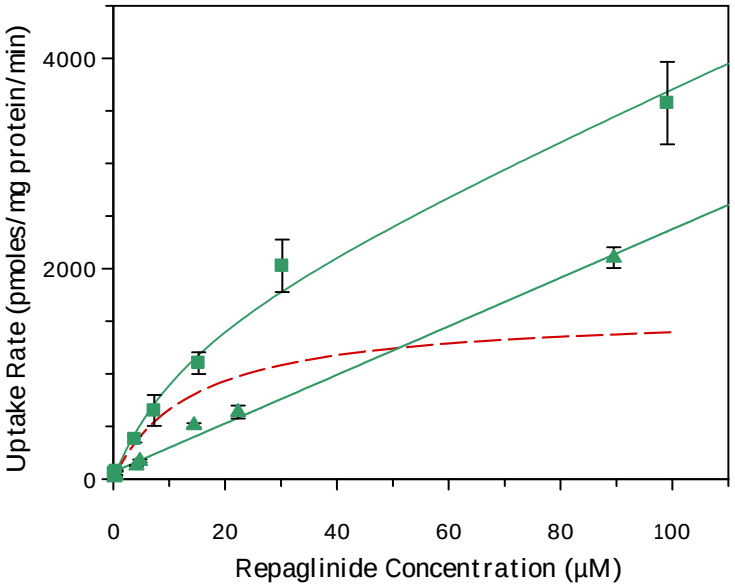
Inhibition of both hepatic uptake and metabolism

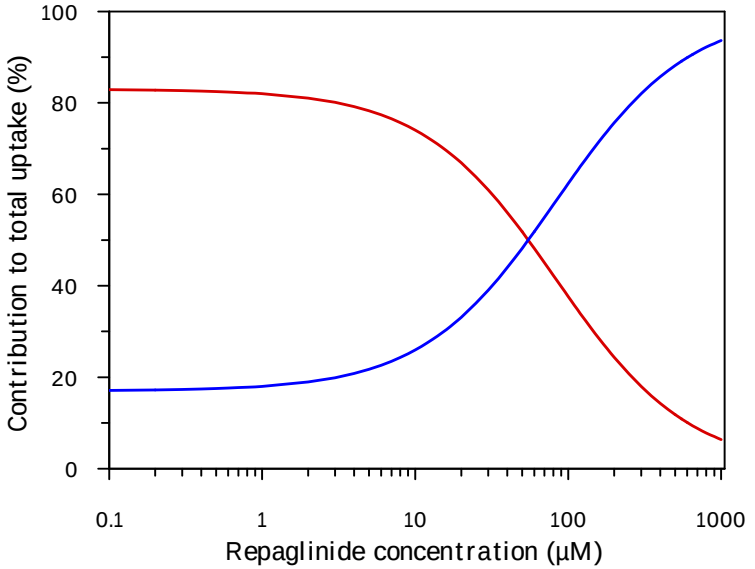


X - inhibition by GFZ and GFZ-glucuronide

DDI at both transporter and P450 level - sequential effect

Repaglinide uptake in human hepatocytes





-■- Total uptake

-▲- Passive component (4°C)

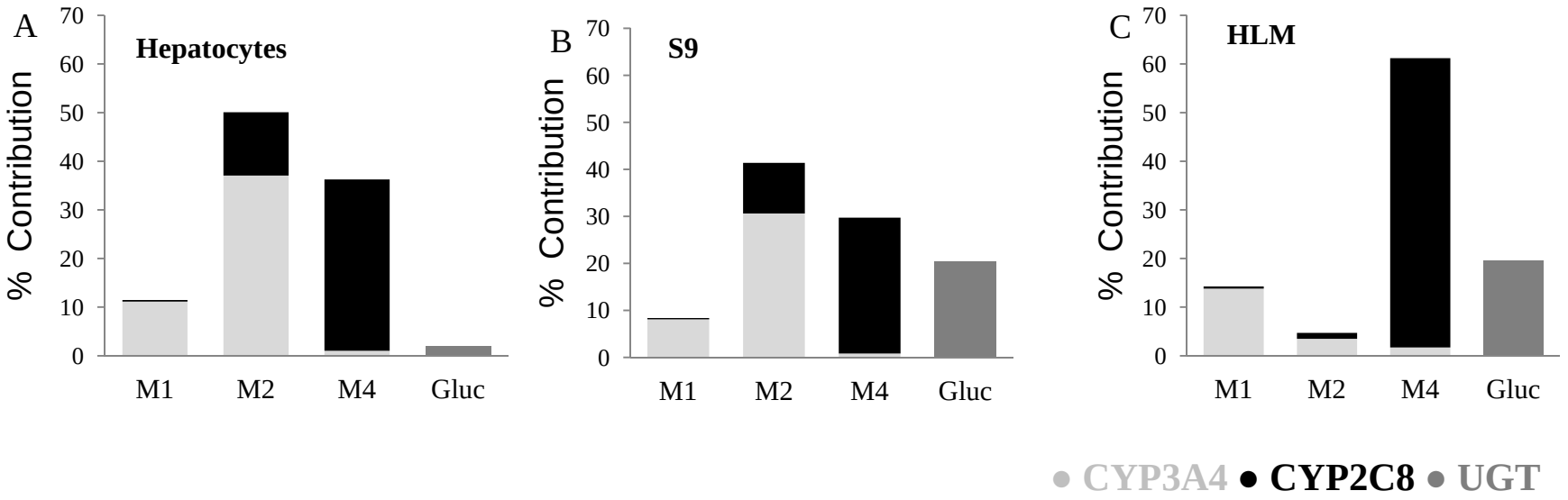
--- Active component (simulated data)

Km = 14.1 µM

CL_{uptake} 5-fold greater than the passive component at therapeutic concentrations

IC50 4.3 and 7.4 µM for GFZ and GFZ-glucuronide, respectively

Comparison of contribution (based on Clints) of pathways across *in vitro* systems

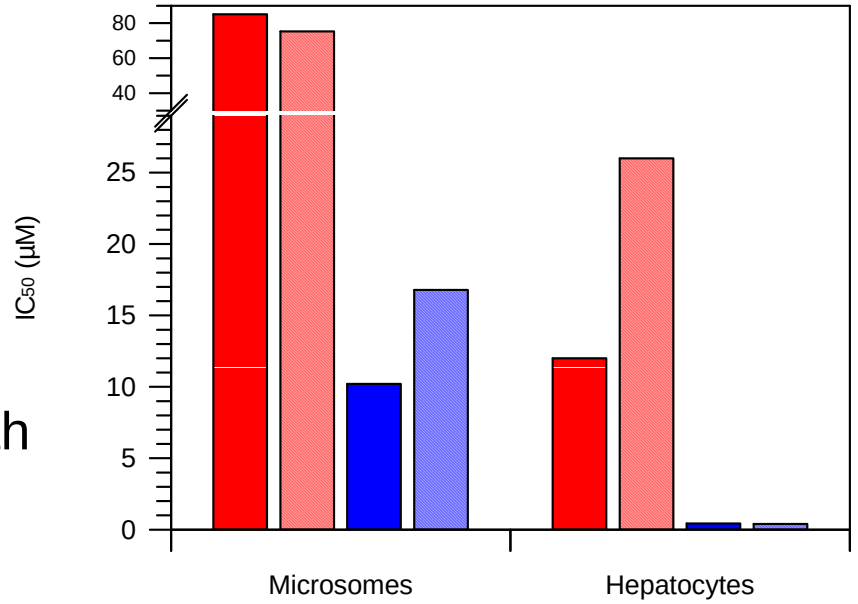


- M2 contributed similar % in S9 and hepatocytes (66% *in vivo*), needs aldehyde dehydrogenase to drive pathway.
- 5% contribution of M2 observed in HLM → increased importance of M1 and M4
- Similar contribution of CYP3A4 and CYP2C8 in S9 and hepatocytes

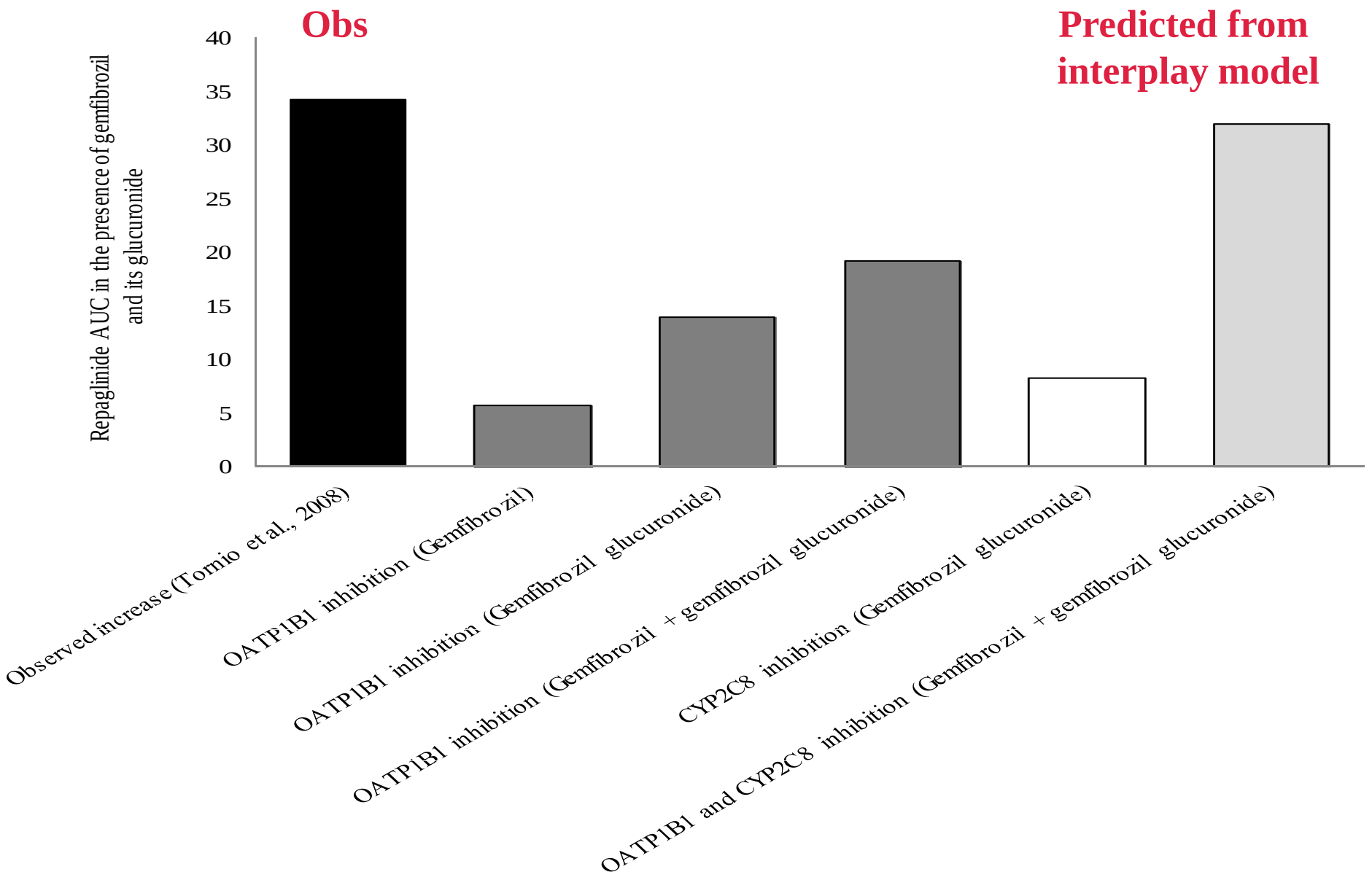
Säll et al, DMD 2012

Inhibition of CYP2C8 by Gemfibrozil *in vitro*

- Using repaglinide depletion and rosiglitazone para-hydroxylation
- Competitive inhibition (red bars) - Microsomal/hepatocyte ratios **7 and 3**
- Time dependant inhibition, with pre-incubation (blue bars) - Microsomal/hepatocyte ratio **24 and 44** [greater accumulation of glucuronide?]



Prediction of Repaglinide-Gemfibrozil DDI



Summary

- Use of rat hepatocytes provides a comprehensive package of clearance mechanisms for PBPK modelling to delineate intracellular events.
- *Kp* parameters particularly useful. Allows resolution of cellular binding and active transport processes.
- Unbound intracellular drug concentration needed – consequences of transporters.
- Certain parameters are translatable to humans (P_{diff}, fu_{cell})

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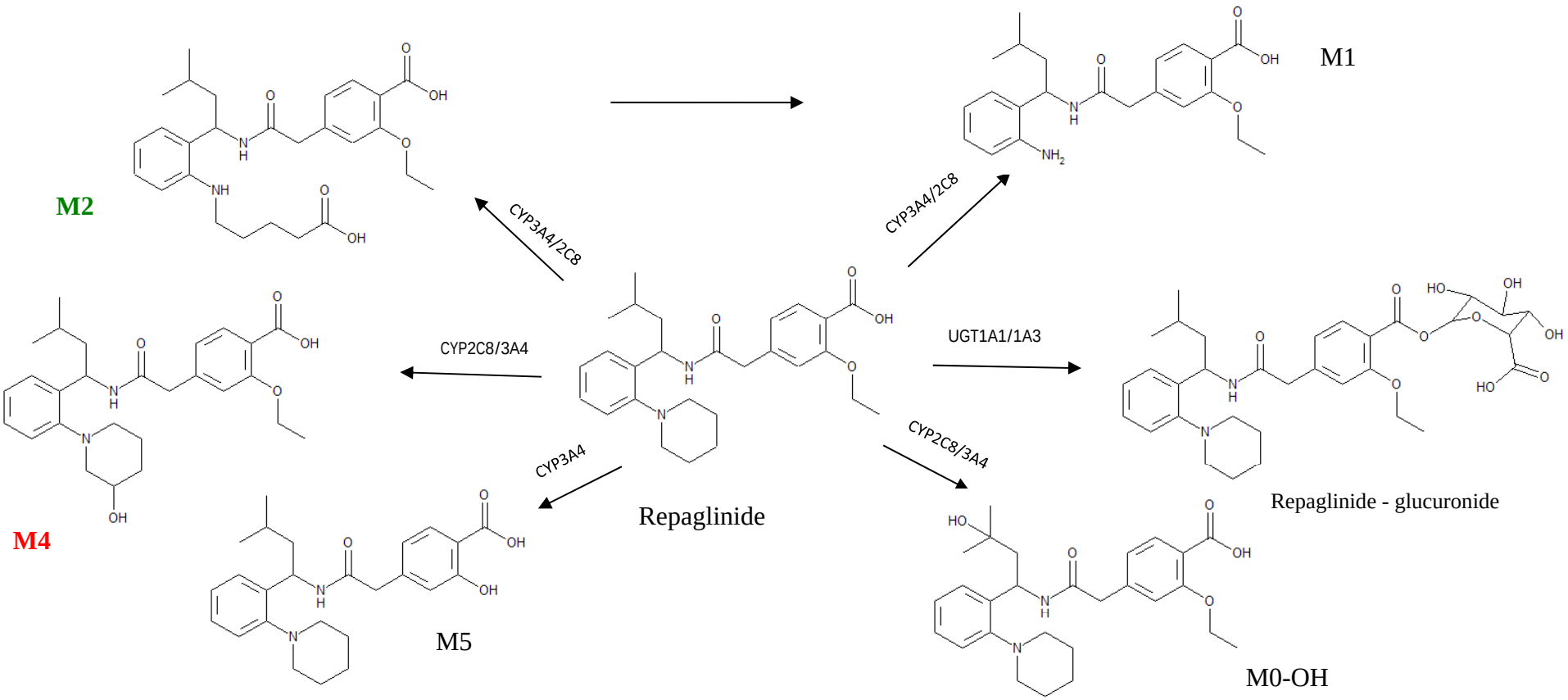
http://www.capkr.manchester.ac.uk

Repaglinide metabolism in human hepatocytes, S9 and microsomes



- Previous *in vitro* study identified **M1** and **M4** as major metabolites
- **M2** reported as major *in vivo* metabolite (66% of dose excreted in faeces and urine)
- No *in vitro* data available to confirm predominant role of CYP2C8 in repaglinide metabolism