Metabolism, Transport, and Mechanistic CL

PSCI-599, Spring 2024

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Clearance

- Removal of drug from system (body)
- Can be through enzymatic metabolism, excretion into urine or bile
  - Drug excreted into bile has chance to reabsorb, remainder excreted in feces
  - Other minor routes include chemical degradation, evaporation & mucociliary CL (ELF), glandular secretion, metabolism by gut bacteria, exsorption
- Mechanistic clearance allows modeling of saturation (enzymes and transporters) and physiologic scaling of intrinsic CL
- In GastroPlus, enzymes and transporters use Michaelis-Menten kinetics, CL_{int} uses linear processes
  - Transporters only move drug from one compartment to another, they do not “remove it from the system”

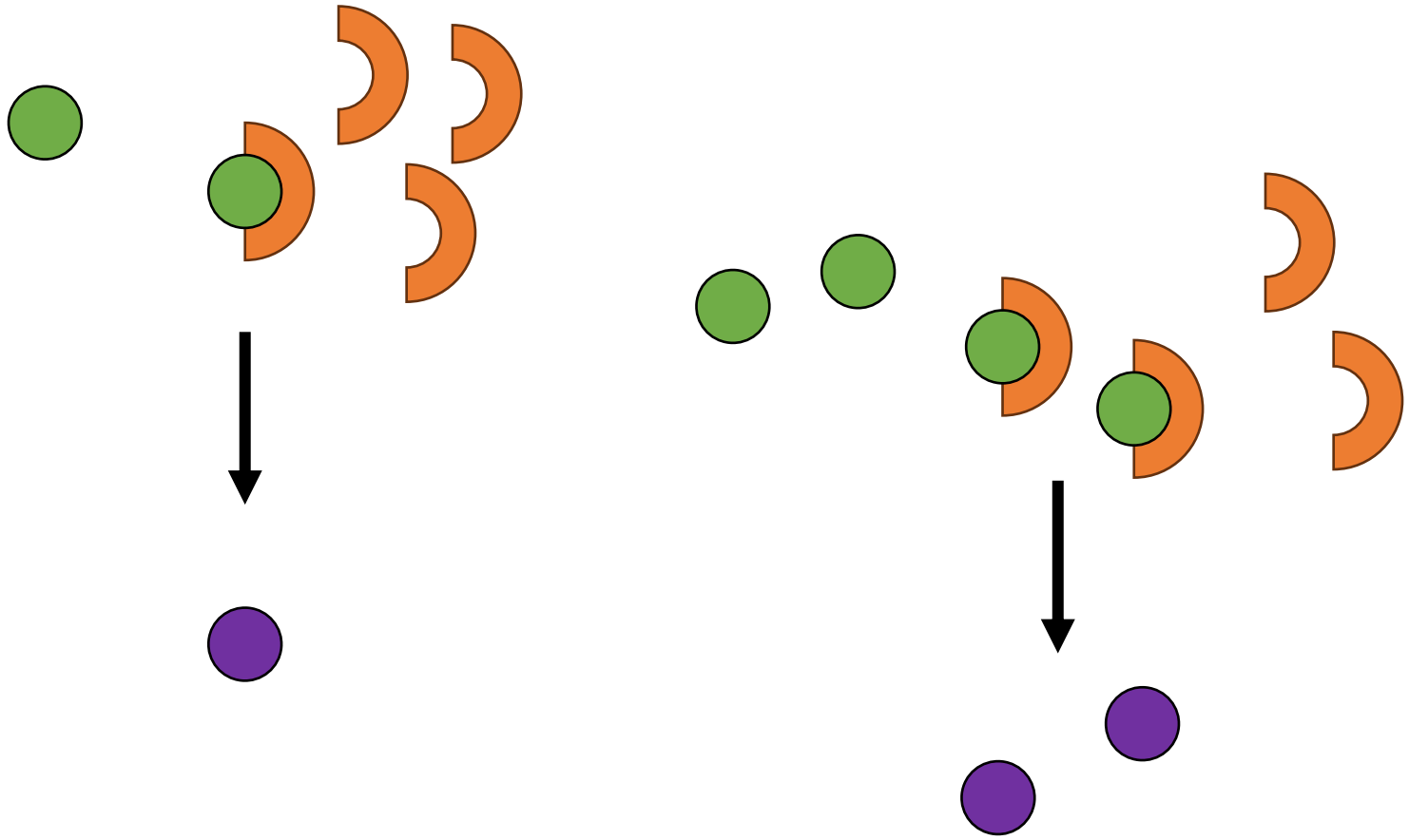
Linear Clearance

Intrinsic Clearance

- Systemic CL and Vd are not drug properties, they are parameters that can be used to describe the pharmacokinetics of a drug *for a specific physiology*
- Intrinsic Clearance (CLint) is the innate ability of a tissue to clear unrestricted drug
  - Units are: volume / time / amount-clearance-mechanism (e.g. mass enzyme)
    - uL/min/nmol 3A4
    - mL/min/mg microsomal protein
- Systemic CL can be derived from CLint(s) by accounting for perfusion (and RBP), protein binding, and permeation to site of clearance
  - CLint will always be higher than systemic CL; systemic CL will always be limited by perfusion

1st order kinetics

$$Rate = \frac{dX}{dt} = -k \cdot X$$

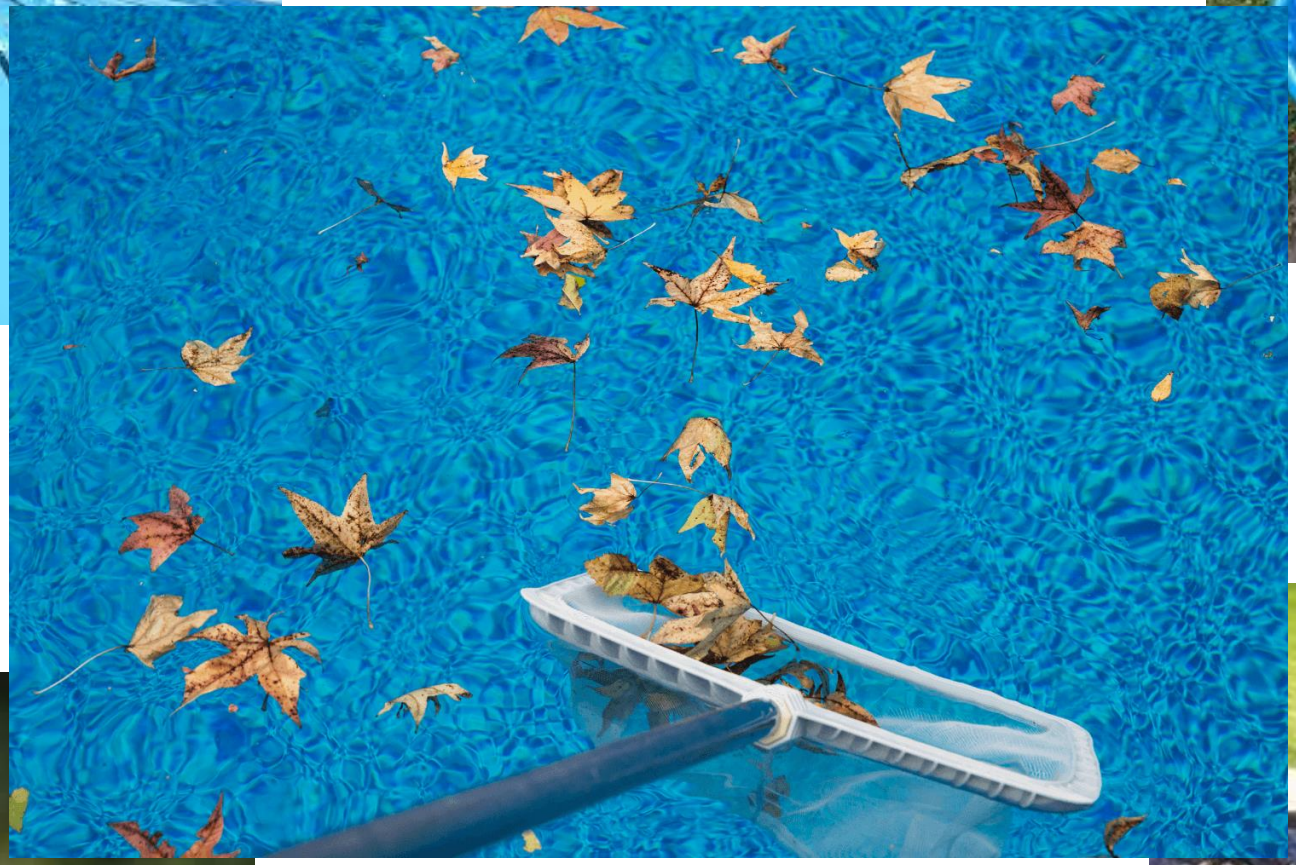


$$\frac{L}{h} \times \frac{mg}{L} = \frac{mg}{h}$$

1st order kinetics have variable reaction rate, but static elimination constant (k) and half-life

1st Order Clearance of Drugs

- Most drugs undergo 1st order (linear) clearance
  - Non-saturated enzymatic metabolism
  - Renal filtration
- Systemic clearance (CL) refers to removal of drug from plasma
  - This is what we can measure
- Units for CL are volume / time
  - Volume of plasma cleared of drug per unit time
    - Concentration * CL = mg/s → Rate varies with concentration, but CL remains constant
- Volume of distribution will affect rate of clearance in terms of drug mass
  - When volumetric flow (CL) is constant, it takes longer to clear a larger volume



Non-Linear Kinetics

Dose (non)proportionality

Dose (mg)	Cmax (ng/mL)	AUC (ng*h/mL)
10	20	100
20	40	200
40	80	400

$$\frac{20}{10} = \frac{40}{20} = \frac{200}{100}$$

$$\frac{40}{20} = \frac{80}{40} = \frac{400}{200}$$

$$\frac{40}{10} = \frac{80}{20} = \frac{400}{100}$$

Dose (mg)	Cmax (ng/mL)	AUC (ng*h/mL)
10	41	285
20	90	571
40	219	1576

Dose (mg)	Cmax (ng/mL)	AUC (ng*h/mL)
10 PO	41	285
20 PO	90	571
40 PO	110	620

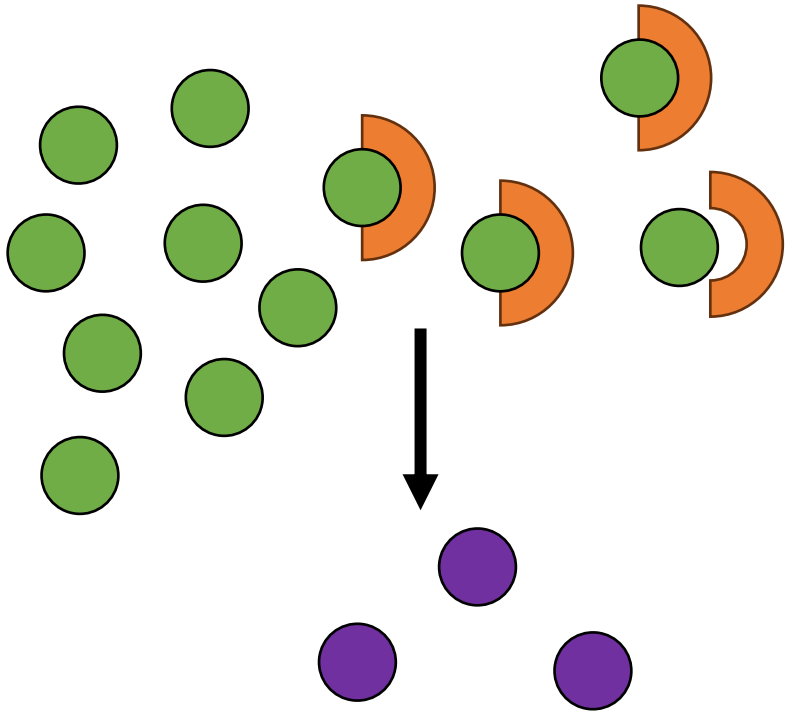
Dose (mg)	Cmax (ng/mL)	AUC (ng*h/mL)
10 IV	32	151
20 IV	63	299
10 PO	2.6	41.5
20 PO	10.2	86.1

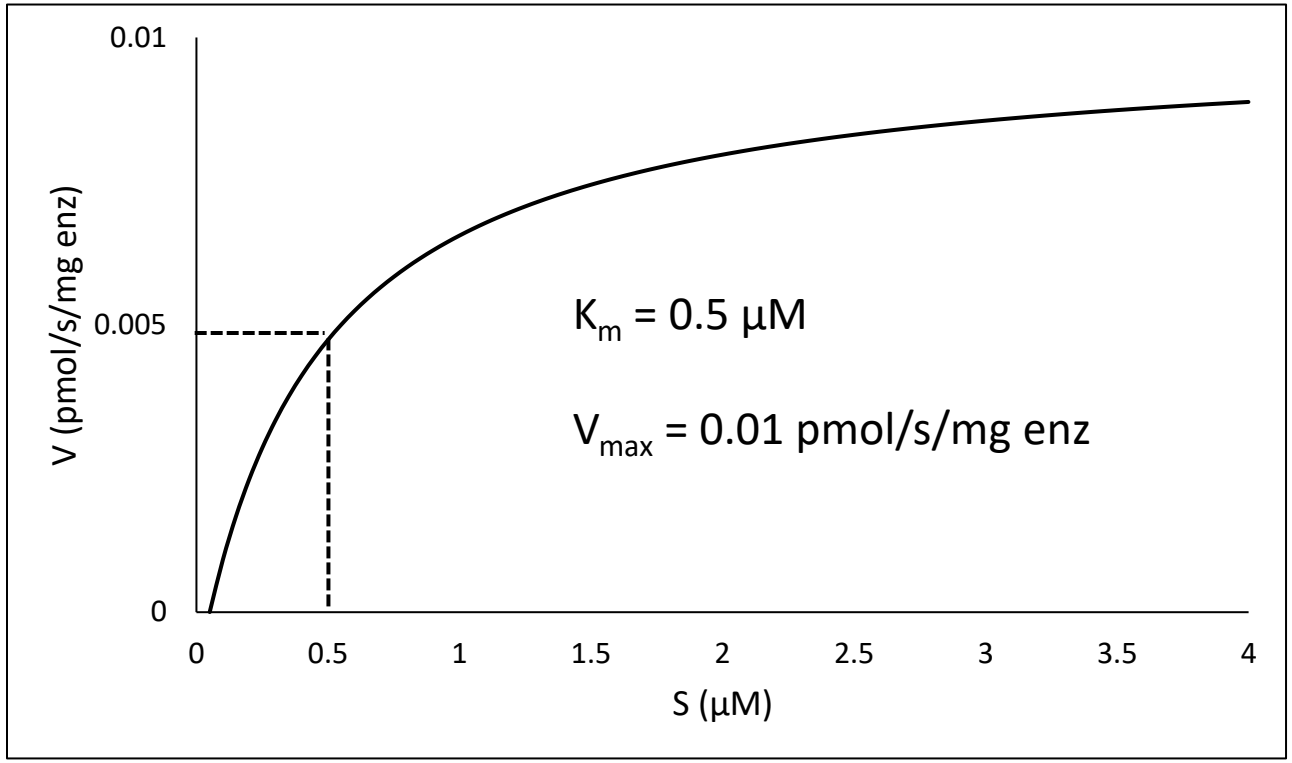
Dose (mg)	Cmax (ng/mL)	AUC (ng*h/mL)
10 IV	21	151
20 IV	39	502
10 PO	5.1	41.5
20 PO	6.9	149

Nonlinear Kinetics

$$V = \frac{V_{max} \cdot [S]}{K_m + [S]}$$

- Nonlinear = Saturable = Michaelis-Menten





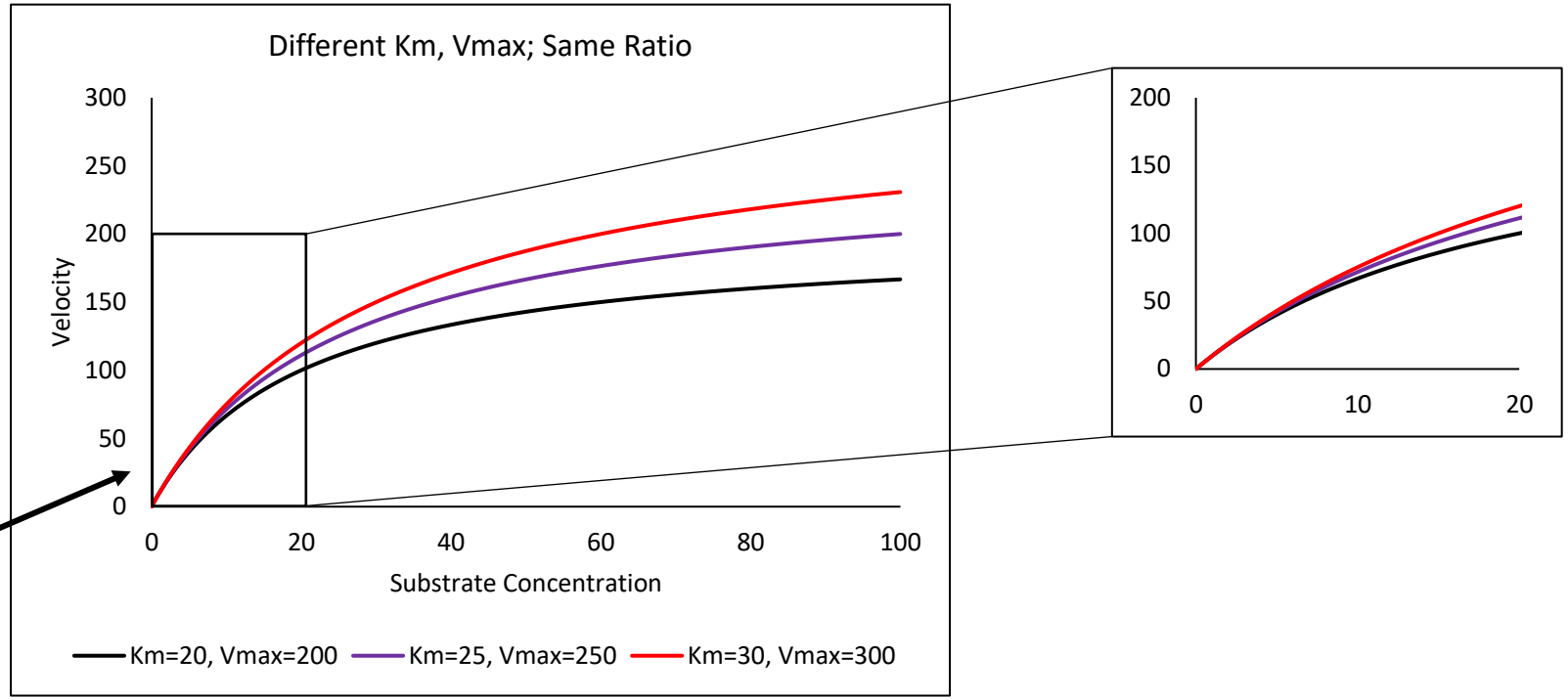
Getting Clearance from Kinetics

$$CL_{int} = \frac{V_{max}}{K_m + [S_U]}$$

When not saturated:

$$CL_{int,in\ vitro} \approx \frac{V_{max}}{K_m}$$

Therefore, can estimate V_{max} from CL_{int} using K_m



Ratio of Vmax:Km behaves similarly for transporters

Effects of Mutations of OAT1

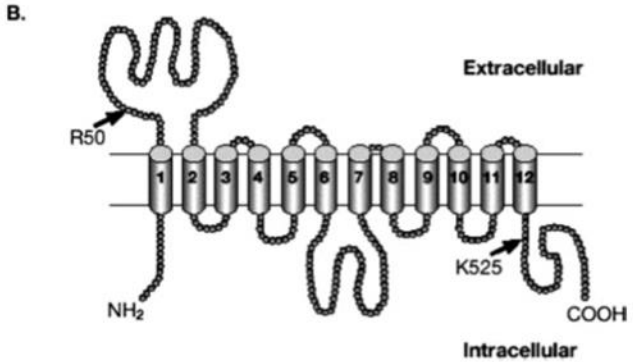
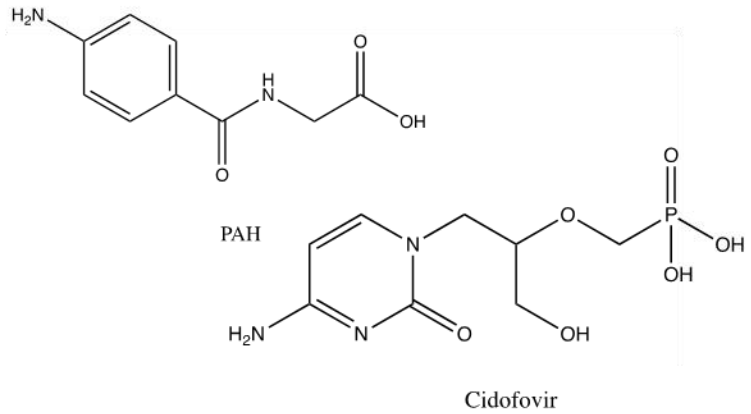


Fig. 1. A, partial alignments of amino acid sequences from human, r and mouse OAT1 and OAT3 in the regions surrounding residues R50 a K525. The conservation of R50 (black shading) and the surrounding mc (gray shading) are indicated. B, predicted secondary structure of hOAT indicating the positions of R50 and K525.



Cidofovir

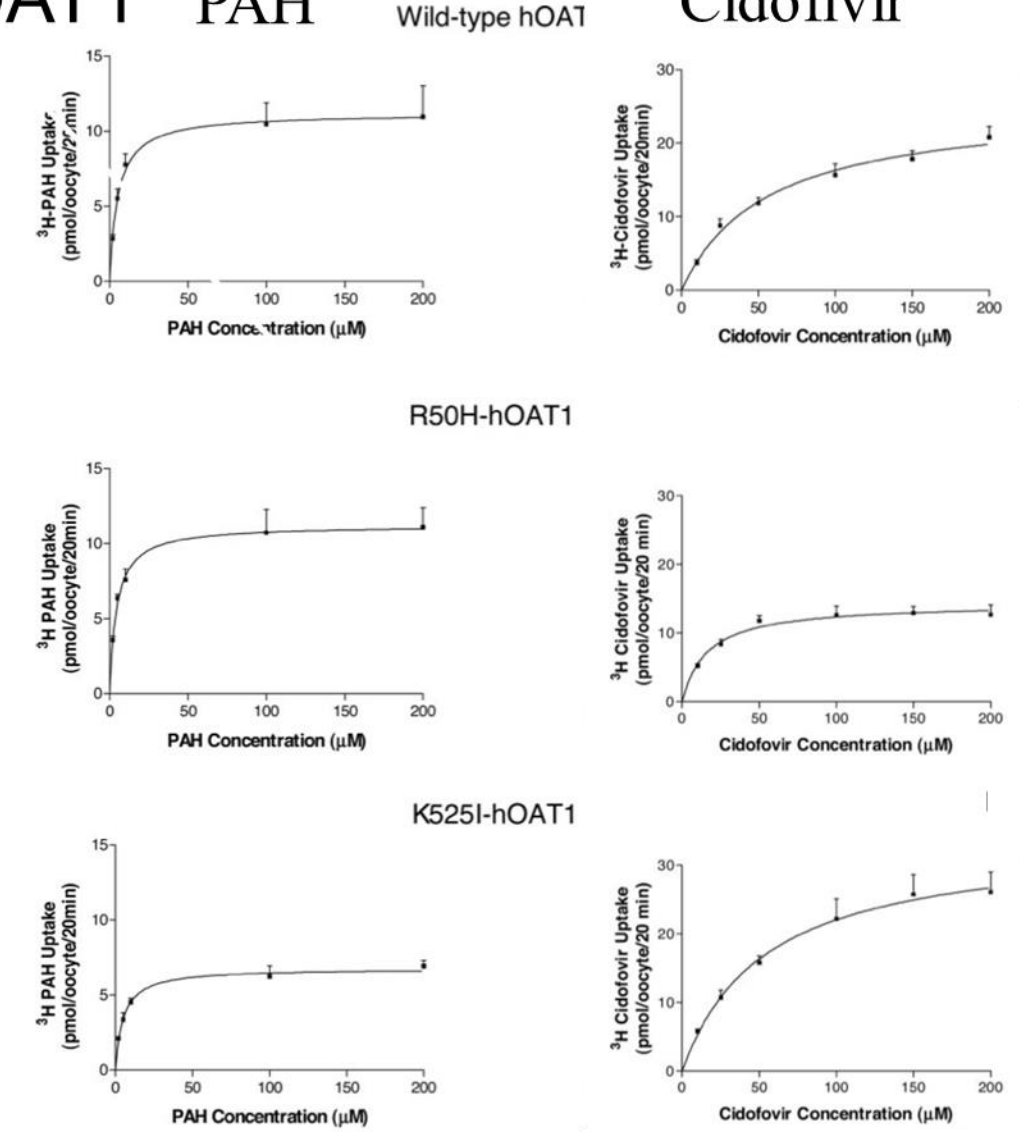
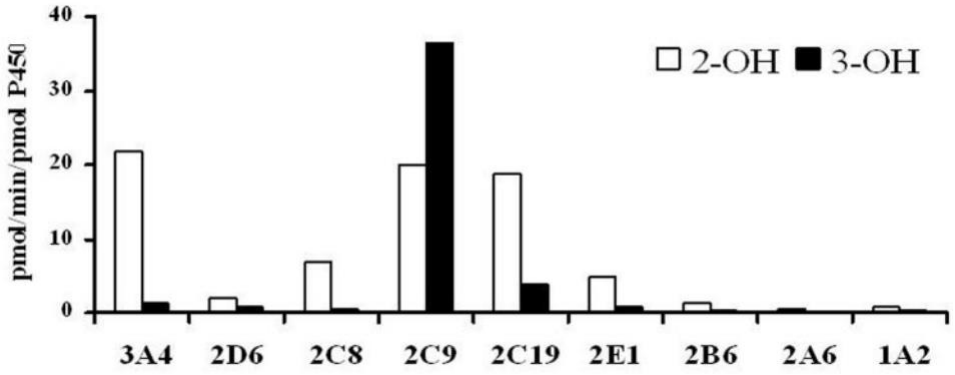


Fig. 2. Representative experiments of PAH transport kinetics for wild-type R50-hOAT1 (K_m = 5.1 ± 0.2 μM, V_{max} = 11.2 ± 0.1 pmol/oocyte/20 min), R50H-hOAT1 (K_m = 4.3 ± 0.1 μM, V_{max} = 11.2 ± 0.1 pmol/oocyte/20 min), and K525I-hOAT1 (K_m = 4.9 ± 0.2 μM, V_{max} = 6.8 ± 0.1 pmol/oocyte/20

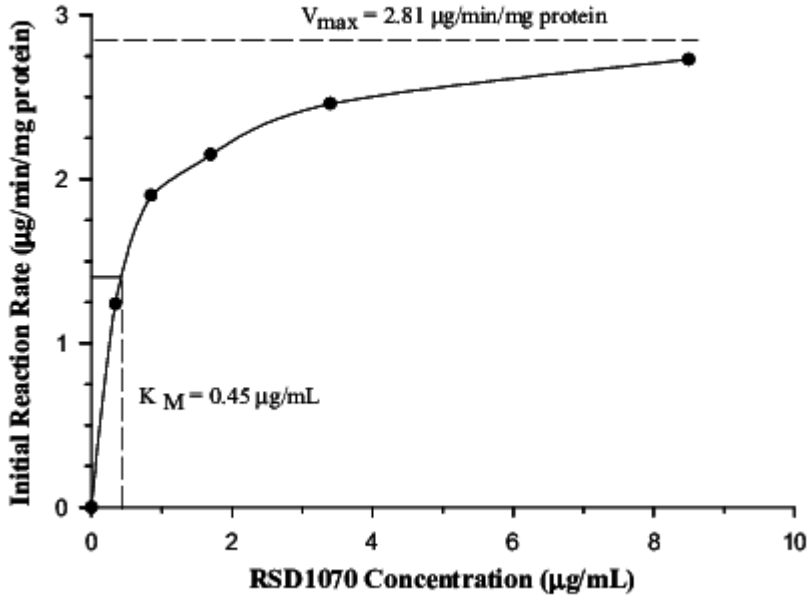
Fig. 4. Representative experiments of cidofovir transport kinetics for wild-type R50-hOAT1 (K_m = 55.4 ± 9.1 μM, V_{max} = 25.3 ± 1.5 pmol/oocyte/20 min), R50H-hOAT1 (K_m = 16.1 ± 2.7 μM, V_{max} = 14.3 ± 0.5 pmol/oocyte/20 min), and K525I-hOAT1 (K_m = 52.3 ± 5.7 μM, V_{max} = 33.6 ± 5.7 pmol/oocyte/20 min).

Bleasby K, et al. *J Pharmacol Exp Ther*. 2005;314(2):923-31.

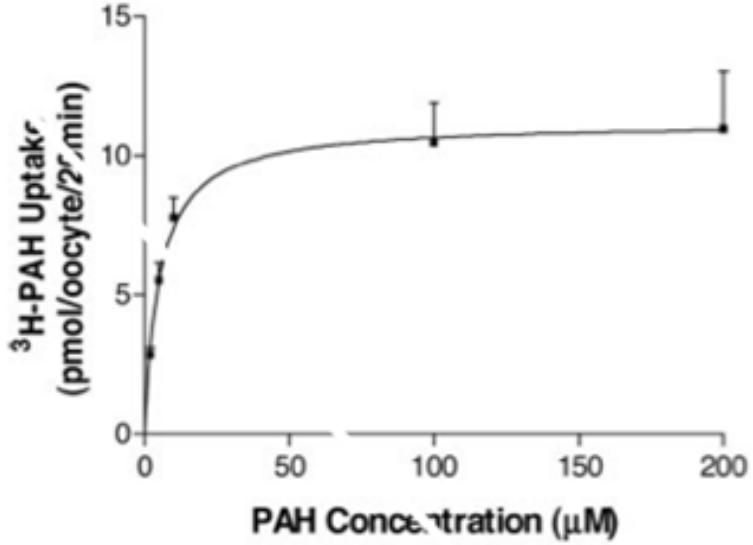
rCYP: per **pmol enzyme**

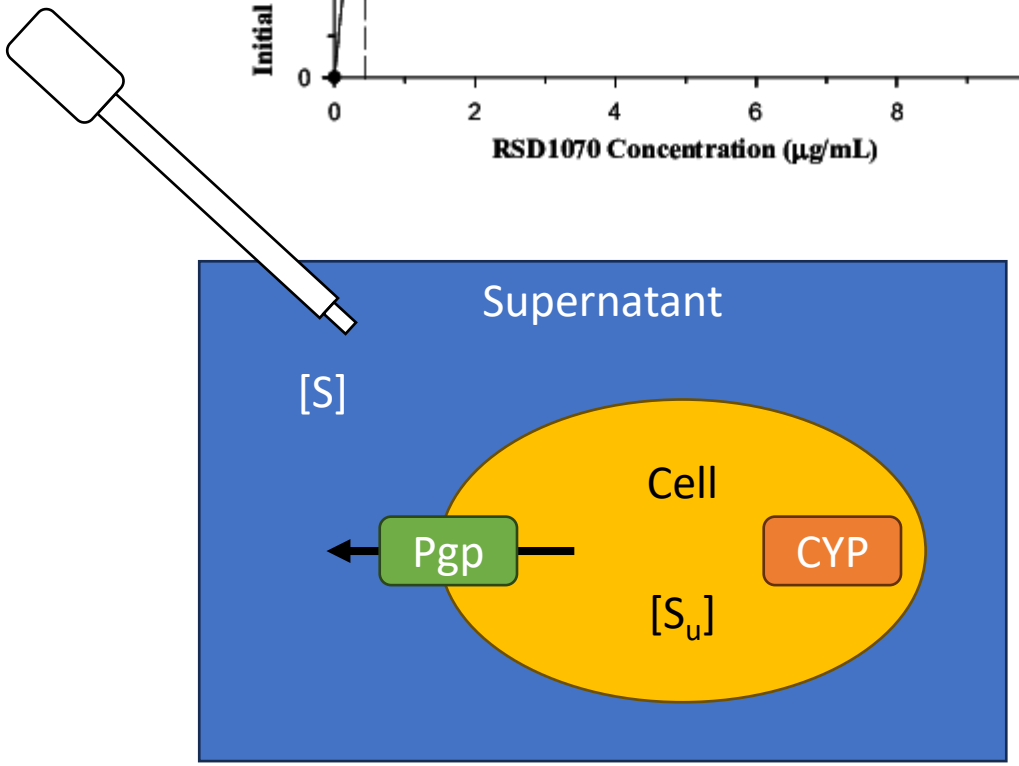


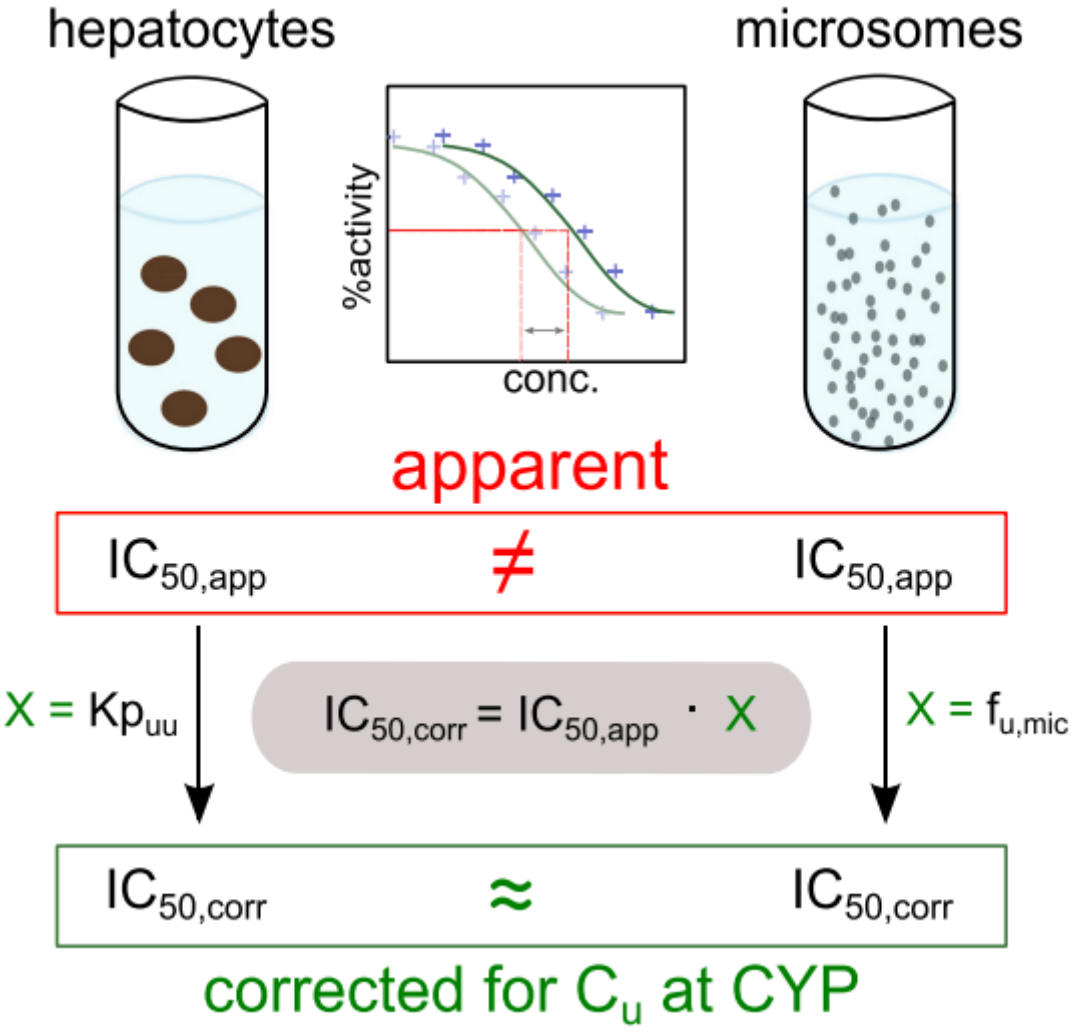
Microsomes: per mg **total** microsomal protein



Expressed Cells/Hepatocytes: per **# cells**





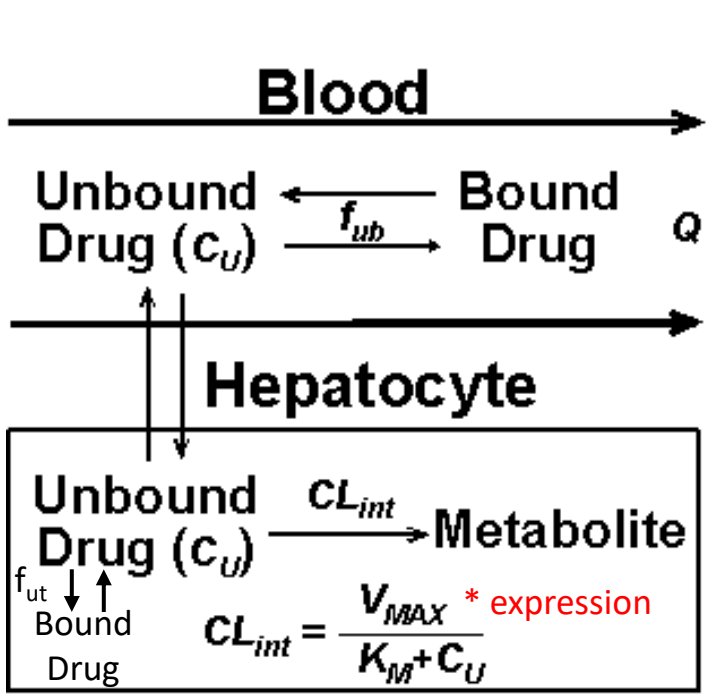


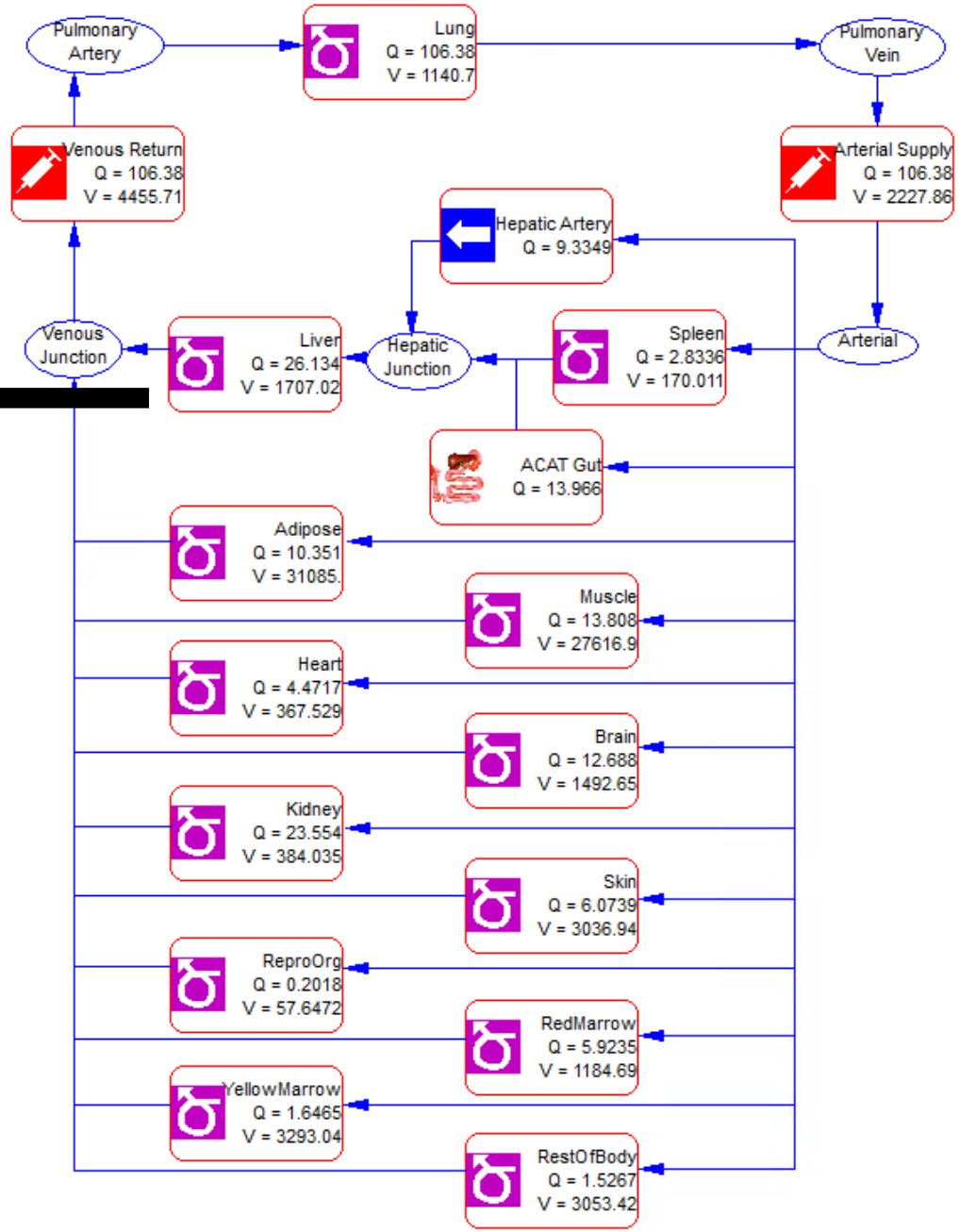
- Different *in vitro* methods give different kinetic parameters

- GastroPlus contains module to convert to *in vivo* kinetics using *in vitro-in vivo* extrapolation (Metabolism & Transporter Module), can also convert *in vitro* CL_{int} to in vivo Cl_{int} **OR** V_{max}/K_m

- GastroPlus further converts units to **mg/s** or **mg/s/mg-enz/trans**
  - Gut uses **mg/s**, using built in scaling for expression
  - PBPK uses **mg/s/mg-enz/trans**, using tissue-specific protein expression

Treyer A, et al. *AAPS Journal*. 2019;21:77.





Same idea for transporters, other tissues

Note that uptake transporters only have an effect in permeability limited tissues

GastroPlus Activities

Demo then Group Work

- Create records for including enzymes/transporters
- Set linear CL to 0 (or proportion of linear CL if multiple routes of elimination), add renal filtration of Fup*GFR
- Add enzymes/transporters to respective tables
  - Make sure to input correct location(s): PBPK +/- Gut only **(no Liver)**
- Use ‘Unit Converter’ to input *in vitro* parameters
- Explore results of nonlinear kinetics on simulations results