SimulationsPlus

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Mechanistic Modeling of *in vitro* Assays to Improve *in vitro/in vivo* Extrapolation

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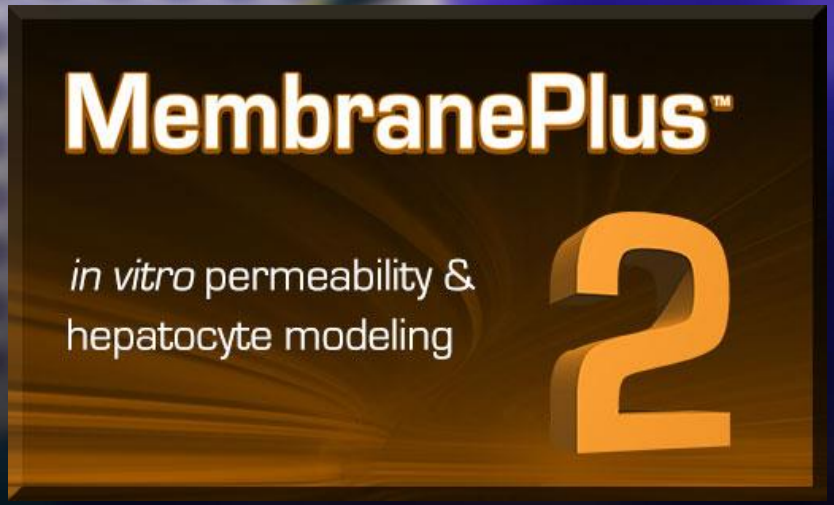
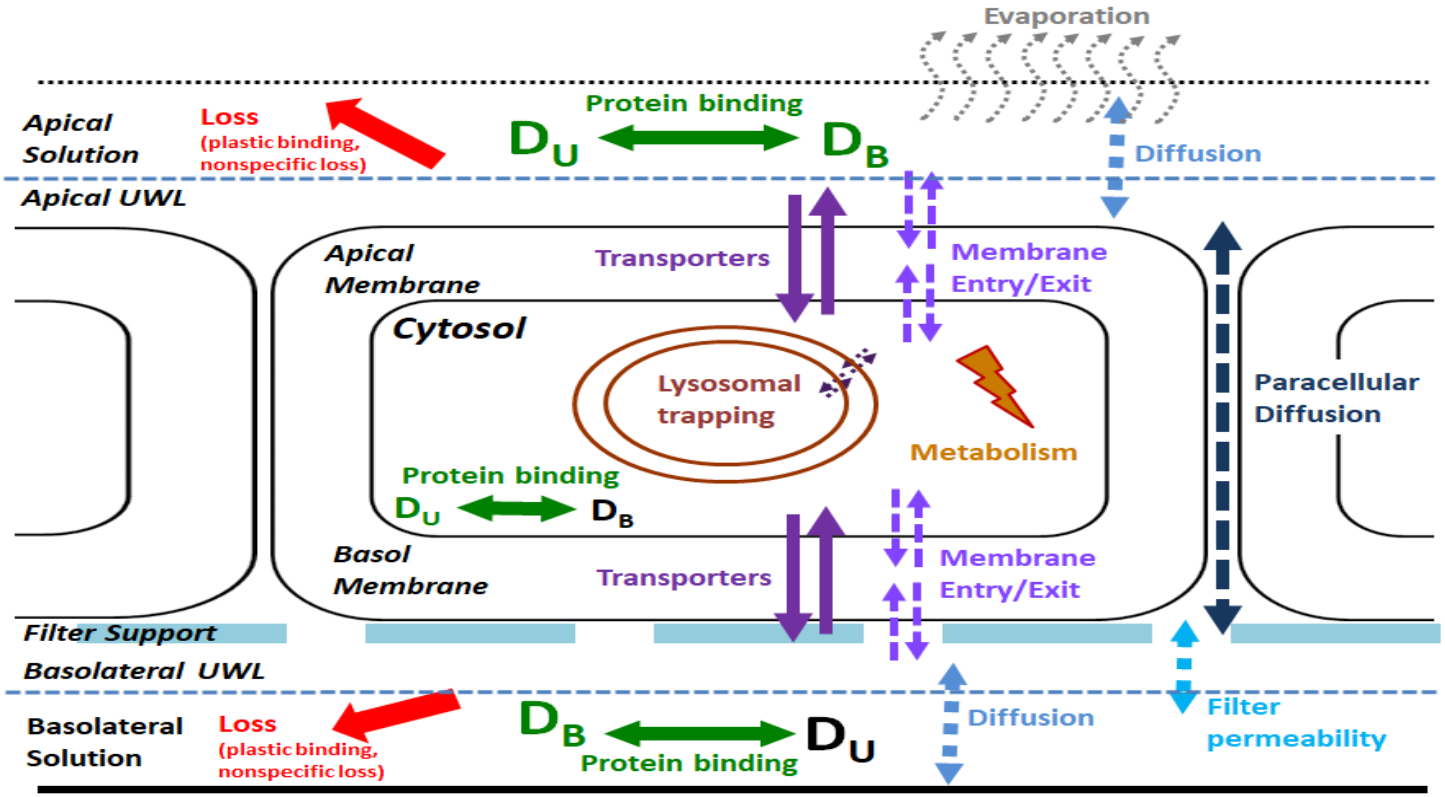
22ND NORTH AMERICAN ISSX MEETING

OVERVIEW

- MembranePlus™ – a software platform for simulation of *in vitro* transport assays:
  - Mechanisms
  - Inputs
  - Models
- Case Studies
  - *In vitro* model validation
  - *In vitro/in vivo* extrapolation



MECHANISMS: TRANSWELL



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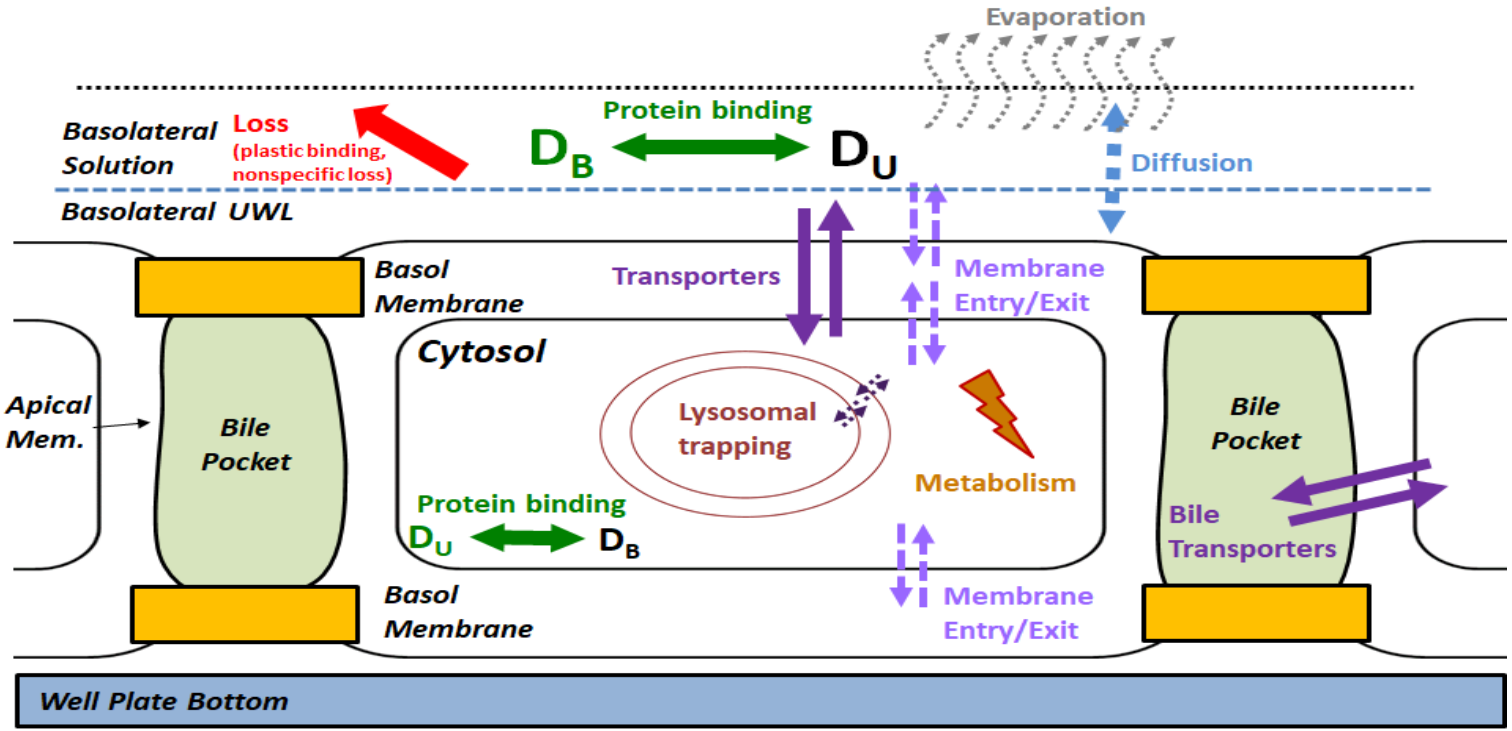
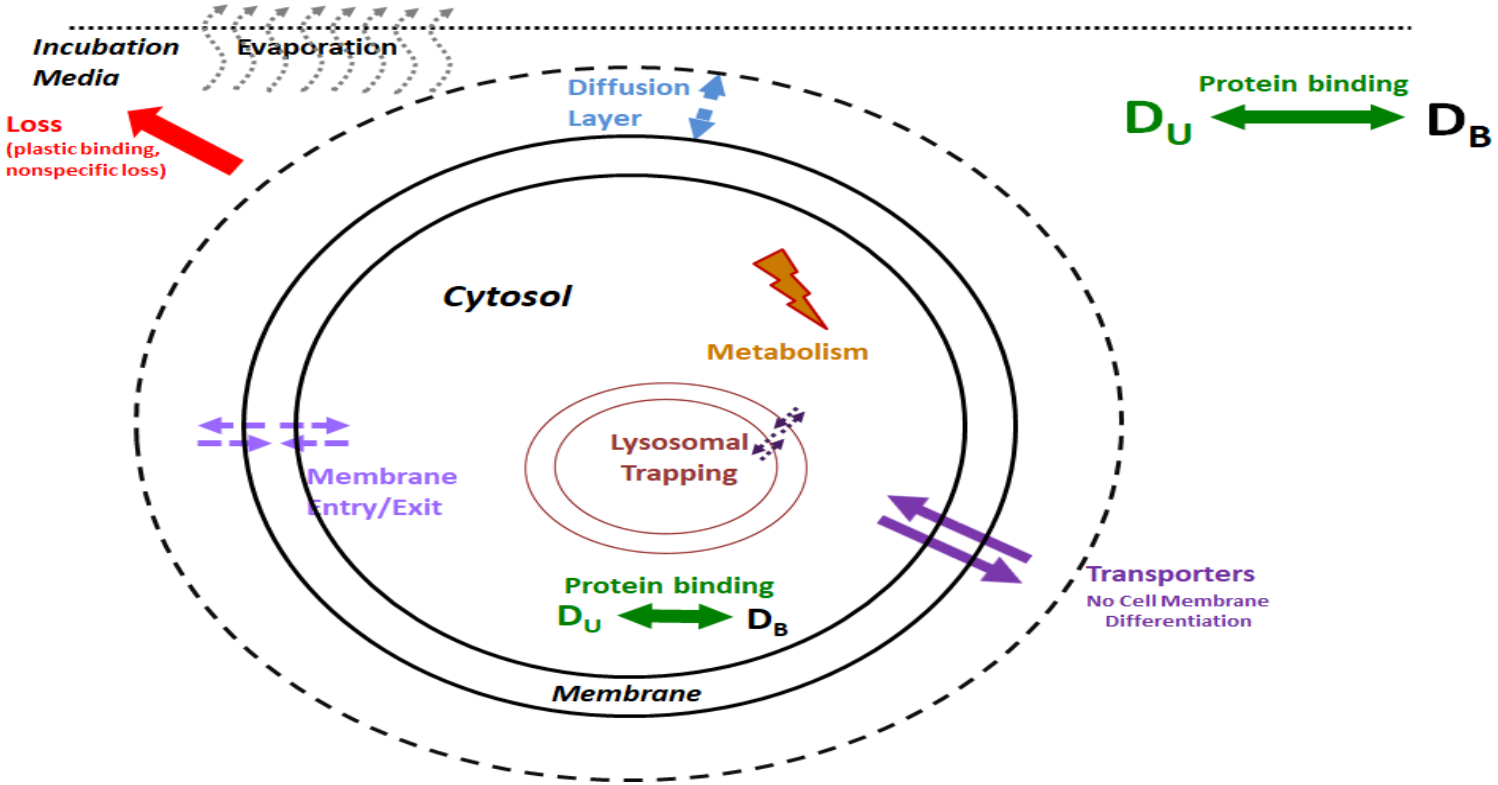
MECHANISMS: SANDWICH HEPATOCYTES



- Collagen is assumed to not affect transport processes
- Model is also applicable for plated hepatocytes when bile volume is not considered

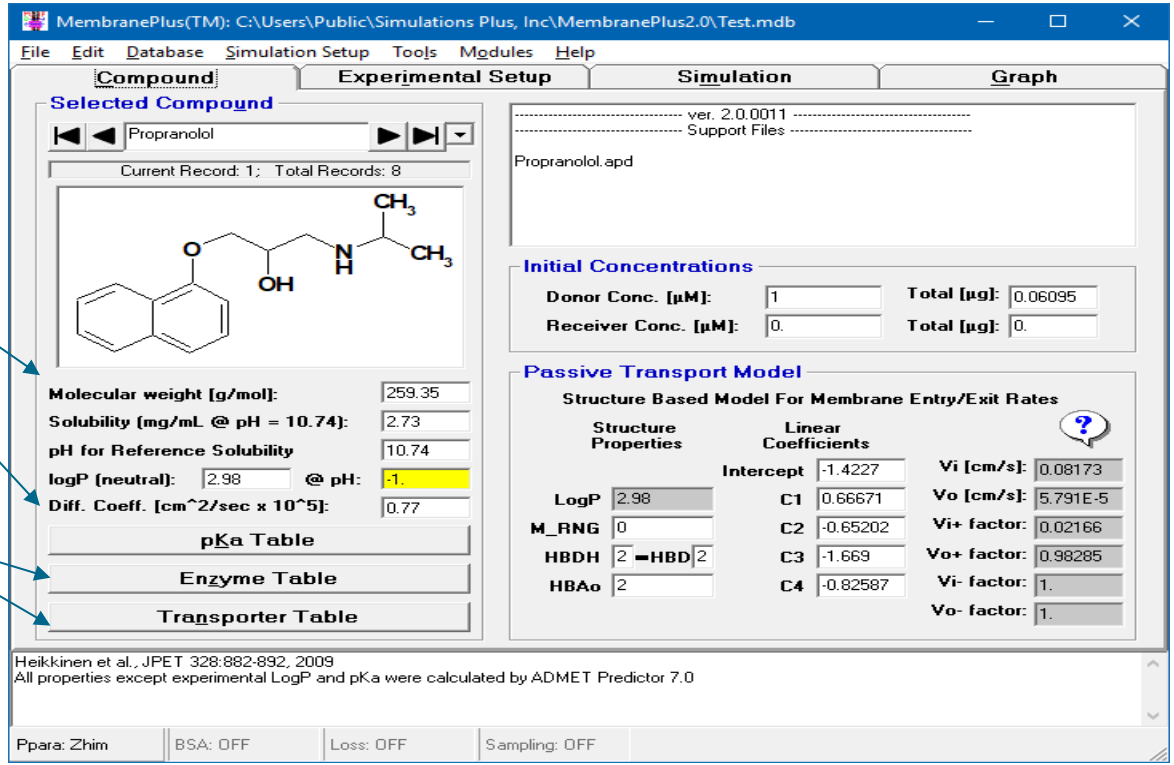
MECHANIMS: SUSPENDED HEPATOCYTES



INPUTS: COMPOUND PROPERTIES

Physicochemical properties (*in vitro* or *in silico*)

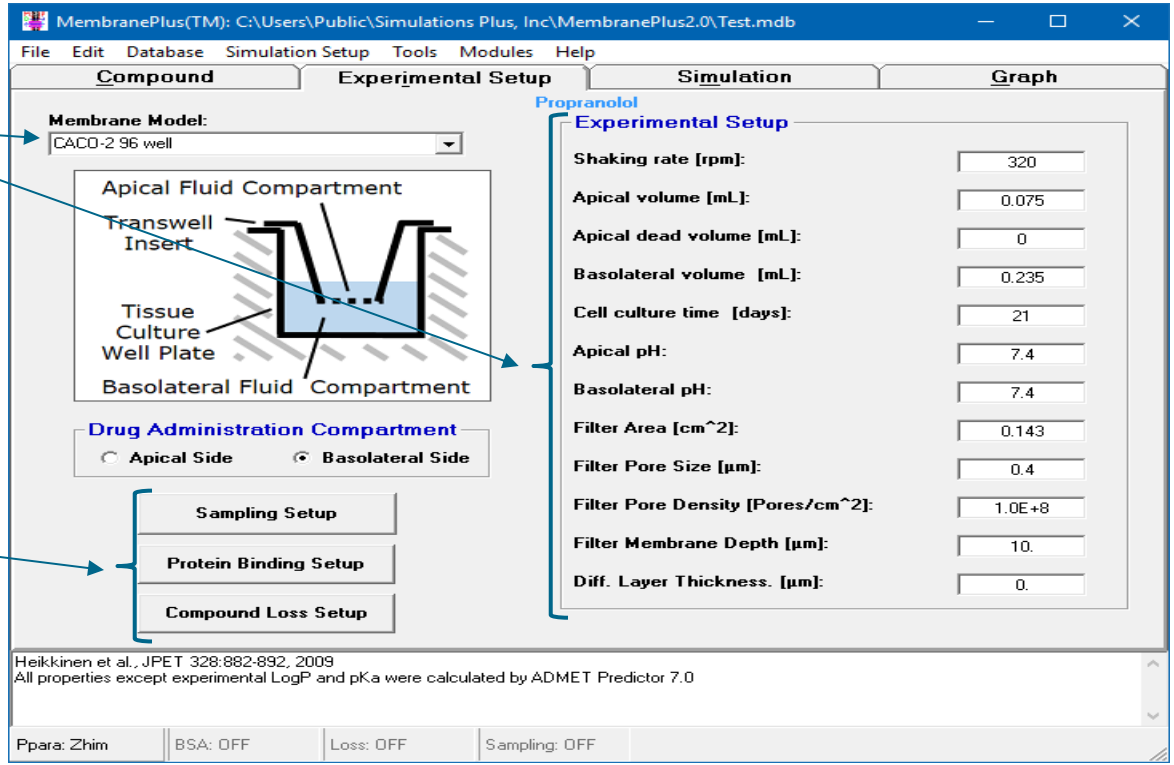
Enzyme and transporter settings applicable only to cell based assays



INPUTS: EXPERIMENTAL SETTINGS

Experimental Setup specific for selected Membrane type (cell monolayers, PAMPA, sandwich hepatocytes, suspended hepatocytes)

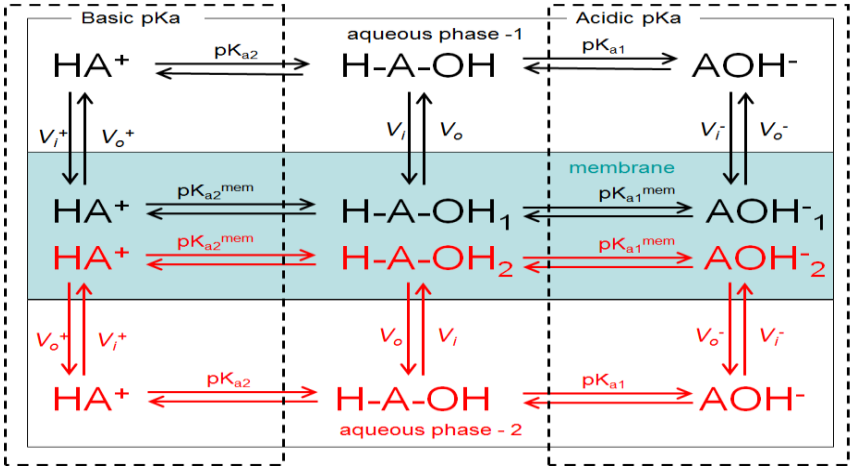
Additional compound/assay specific settings



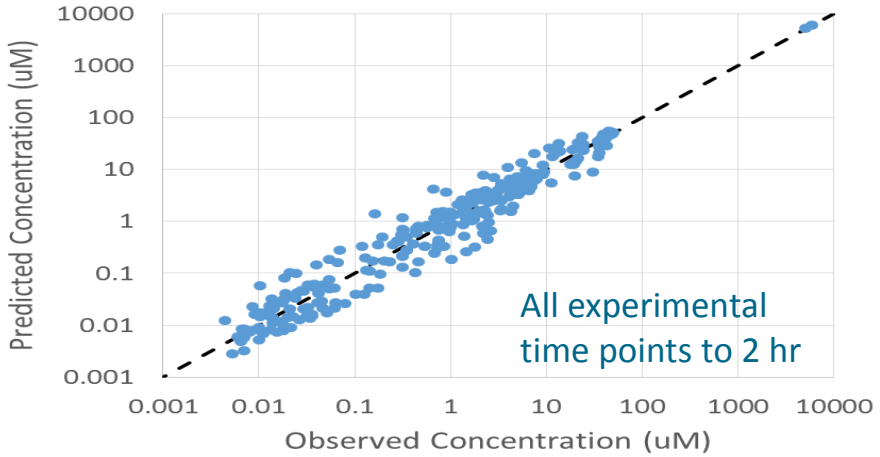
MODELS: MEMBRANE ENTRY/EXIT RATE STRUCTURE BASED MODEL

- Observed vs. Predicted on 44 training datasets

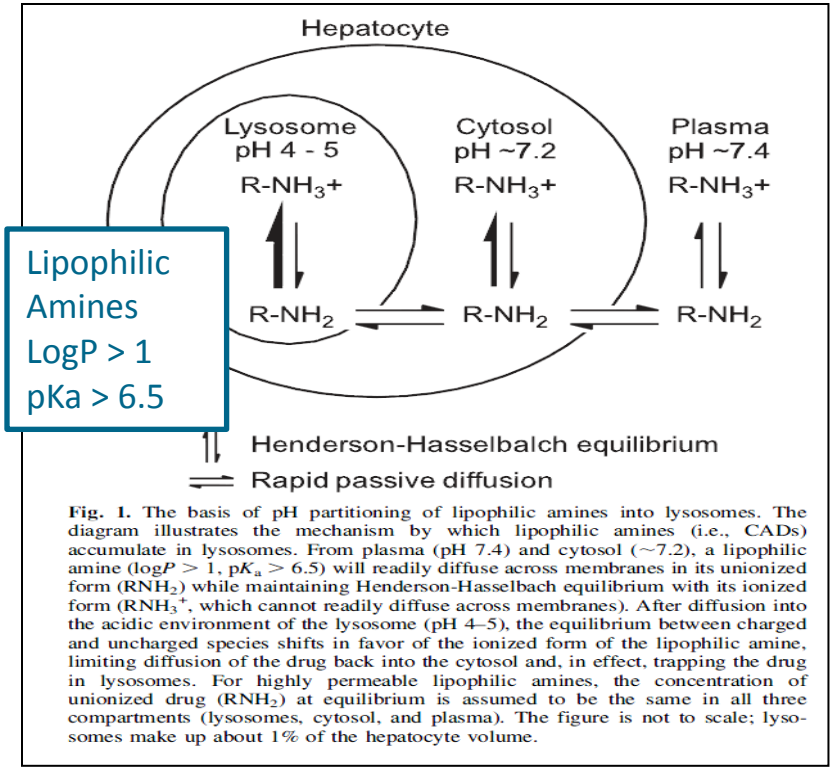
$$log(V_i) = Intercept + C1 \times logP + C2 \times M\_RNG + C3(HBDH - HBD) + C4 \times HBAo$$



Membrane entry and exit rates for anions and cations are determined based on logD vs. pH profile

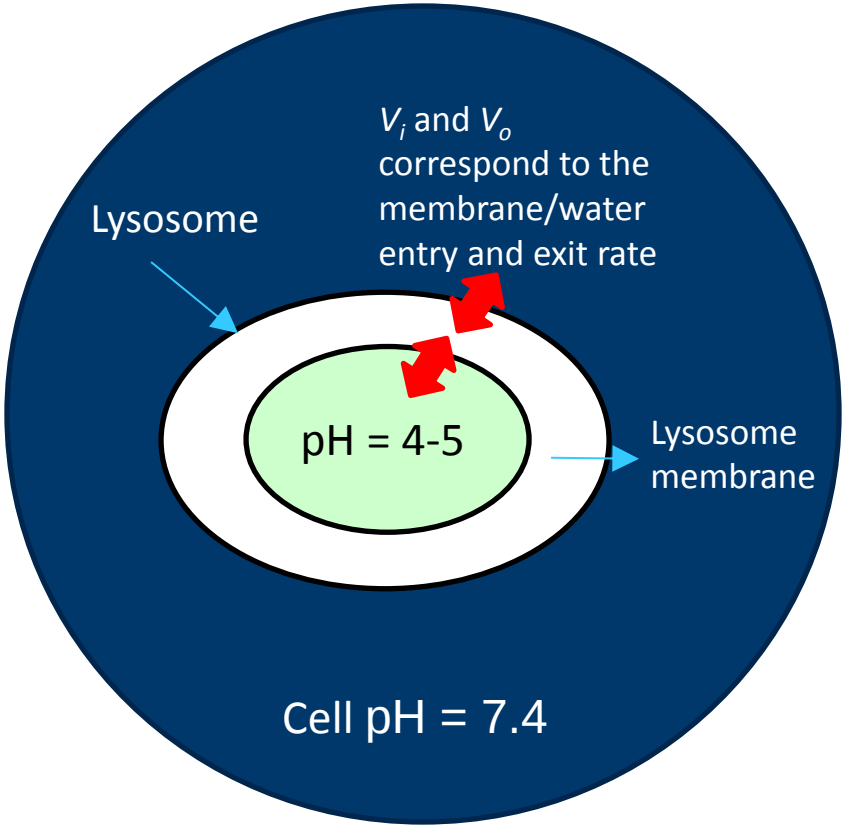


MODELS: LYSOSOMAL TRAPPING



Lipophilic Amines
LogP > 1
pKa > 6.5

Fig. 1. The basis of pH partitioning of lipophilic amines into lysosomes. The diagram illustrates the mechanism by which lipophilic amines (i.e., CADs) accumulate in lysosomes. From plasma (pH 7.4) and cytosol (~7.2), a lipophilic amine ($\log P > 1$, $pK_a > 6.5$) will readily diffuse across membranes in its unionized form (RNH_2) while maintaining Henderson-Hasselbach equilibrium with its ionized form (RNH_3^+, which cannot readily diffuse across membranes). After diffusion into the acidic environment of the lysosome (pH 4–5), the equilibrium between charged and uncharged species shifts in favor of the ionized form of the lipophilic amine, limiting diffusion of the drug back into the cytosol and, in effect, trapping the drug in lysosomes. For highly permeable lipophilic amines, the concentration of unionized drug (RNH_2) at equilibrium is assumed to be the same in all three compartments (lysosomes, cytosol, and plasma). The figure is not to scale; lysosomes make up about 1% of the hepatocyte volume.



MODELS: ENZYMES AND TRANSPORTERS

- Kinetics of carrier-mediated transport and metabolism is calculated using Michaelis-Menten kinetics:

$$u_{metab} = \sum_i \left(\frac{V^i_{\max} \times c_u^{\text{intracell}}}{K^i_m + c_u^{\text{intracell}}} \right) \qquad \upsilon_{efflux} = \sum_i \left(\frac{V^i_{\max} \times c_u^{\text{intracell}}}{K^i_m + c_u^{\text{intracell}}} \right)$$

$$\upsilon_{influx} = \sum_i \left(\frac{V^i_{\max} \times c_u^{buffer}}{K^i_m + c_u^{buffer}} \right)$$

- V_{max} units: µM/s (mmol/L/s)
- K_m units: µM (mmol/L)
- General units converter allows converting these into different types of units
- Transporter types: Influx and Efflux
- Transporter locations: Apical and Basolateral (where applicable)

Case Studies: *in vitro* Model Validation

Case Study 1: Sodium Taurocholate Uptake into Bile

SODIUM TAUROCHOLATE

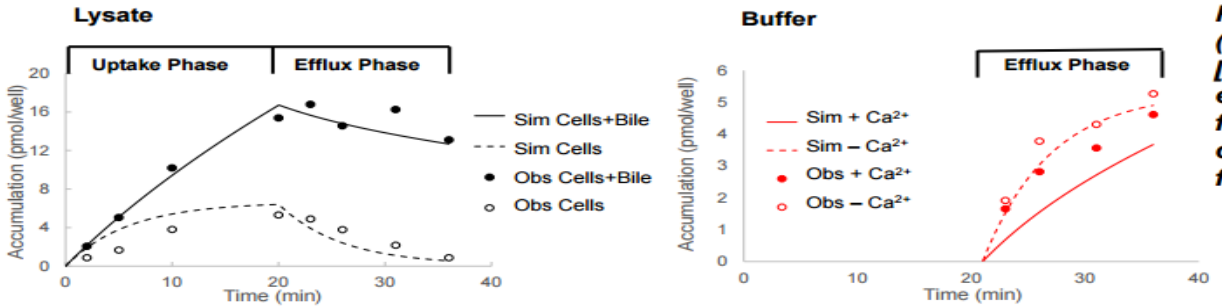
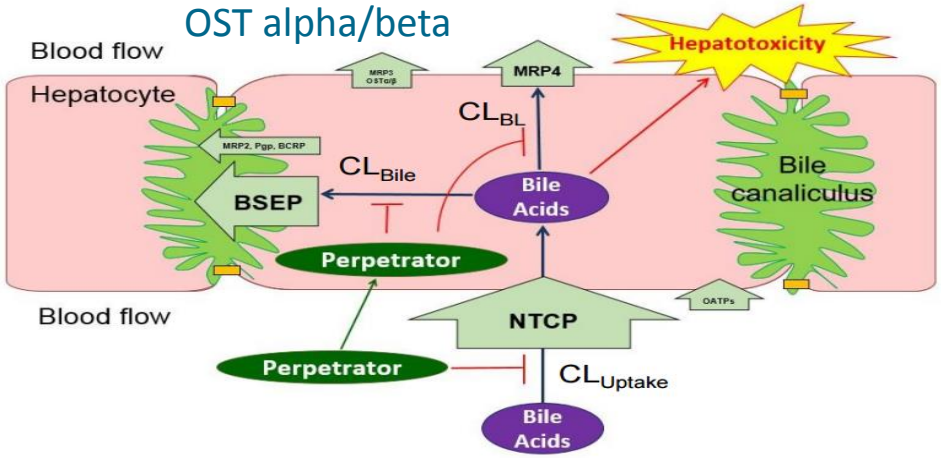


Fig. 3. Observed (circles) and simulated (lines) d_8-TCA accumulation in lysate [from cells+bile and cells] (black) and efflux buffer [Ca^{2+}-containing and Ca^{2+} -free] (red). Representative graph from one of n=3 experiments. Recovered estimates for CL (mean and SD):

(µL/min/mg protein)	Mean	SD
CL_{Uptake}	6.97	1.39
CL_{BL}	0.379	0.12
CL_{Bile}	0.827	0.30

Guo, ISSX 2014

OST alpha/beta



MEMBRANEPLUS MODEL ASSUMPTIONS

- Assumptions
  - No protein binding for Na Taurocholate
  - No stirring
  - Complete monolayer of cells (100% viability)
  - ADMET Predictor values for properties and transport model parameters calculated using calibration

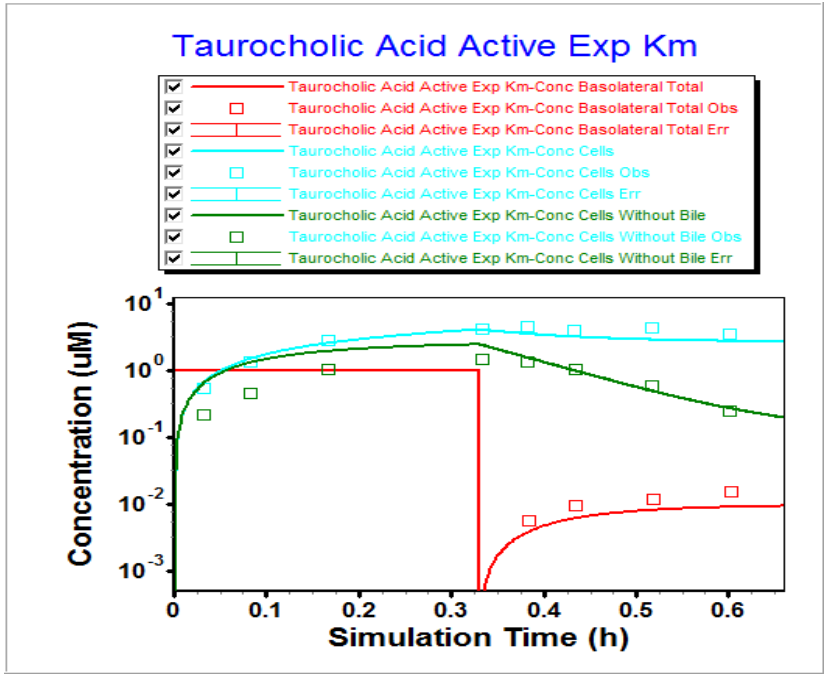
Cell Assay Inputs		
Feed Solution Conc.	1,2.5	µM
BSA	4	%
Well size	24	well
Volume	0.3	mL
Cell Vol.	6.46	pL
Cell Layer Thickness	18.6	micron
Cell Den	0.4	Mcell/well

- Literature values for K_m were used as a starting point for building the model.
- K_m values are similar across species
  - Swift-Mol-Pharm-2010-7(2)-491–500

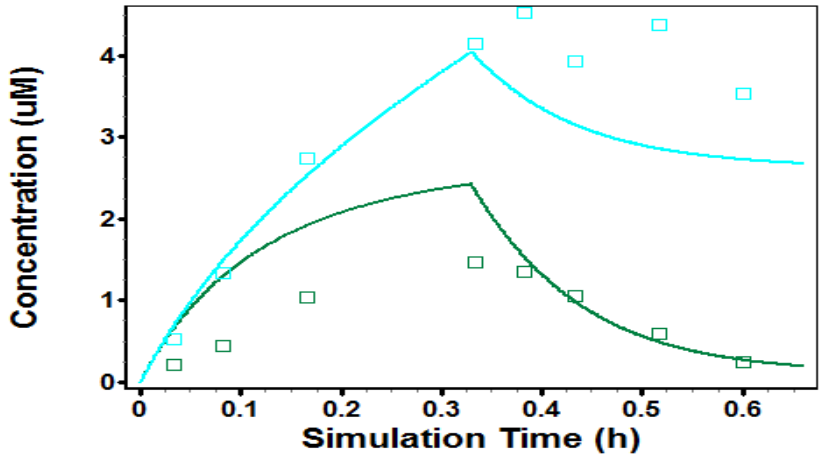
Transporter	K_m (µM)	Cells	Literature Source
OST alpha/beta	25.8	Human	Swift-Mol-Pharm-2010-7(2)-491–500
Overall Uptake	19	Rat	Schwarz-Eur-J-Biochem-1975-55-617-623
NTCP	6	Human	J-Exp-Biol-2001-204-1673-1686
BSEP	5	Rat	J-Exp-Biol-2001-204-1673-1686

SIMULATION RESULT

- Experimental K_m values utilized from literature and V_{max} values fit to data (all remaining properties were predicted by ADMET Predictor).



Transporter	Type	K_m (Exp) (µM)	V_{max} (µmol/s/L)
BSEP	Efflux	5	6.83E-03
OST alpha/beta	Efflux	25.8	9.63E-02
NTCP	Influx	6	3.88E-02



Case Study 2: Quantification of Influx Transport vs. Metabolism Statin Compounds

Suspended Hepatocytes

QUANTIFY THE RELATIVE IMPORTANCE OF INFLUX TRANSPORT (OATP1B1) VS. METABOLISM

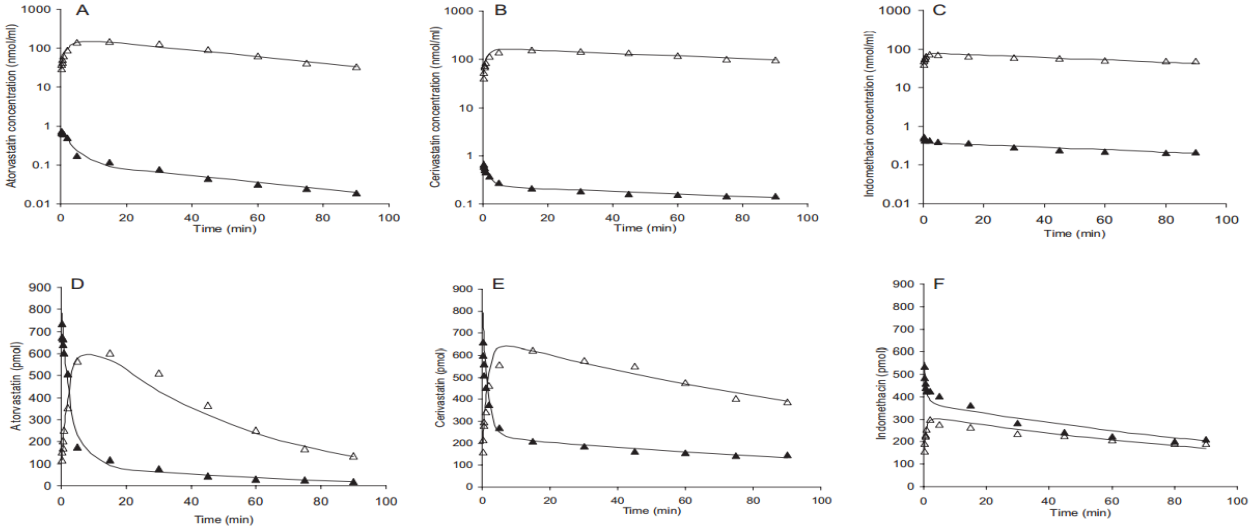


FIG. 3. Typical plots for atorvastatin (A and D), cerivastatin (B and E), and indomethacin (C and F) cell and medium data and associated WinNonlin-generated fits to the model (Fig. 1). A to C, concentration of drug in cells and medium; D to F, amount of drug in cells or medium: cells (△, cells; ▲, medium).

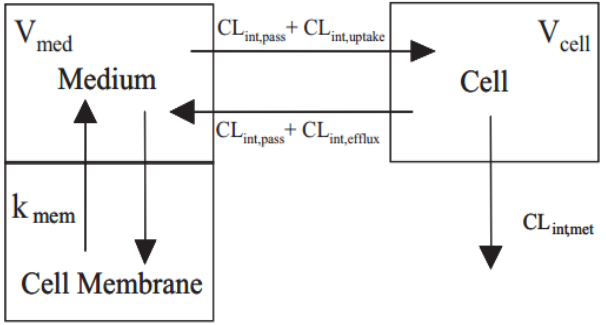
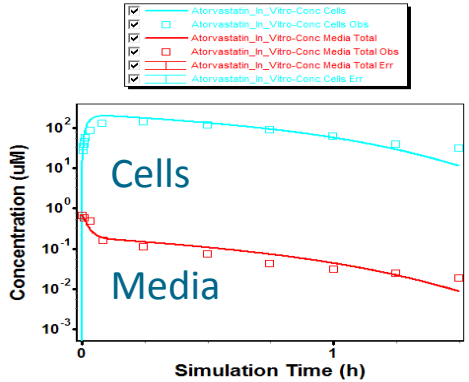


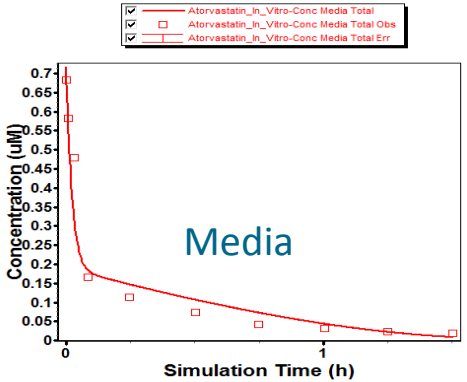
FIG. 1. Compartmental model describing hepatocyte incubation.

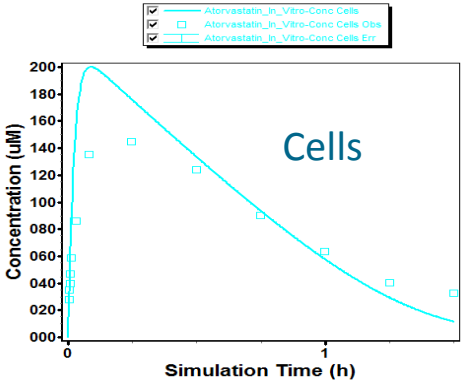
- Media and whole cell concentration data for atorvastatin, cerivastatin, and indomethacin.
- Used a simple compartmental model to extract clearance values

MEMBRANEPLUS MODEL RESULTS I

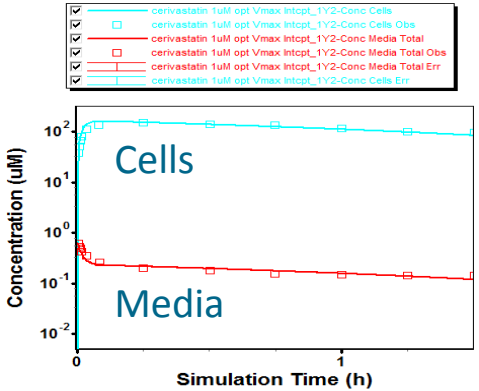
Atorvastatin

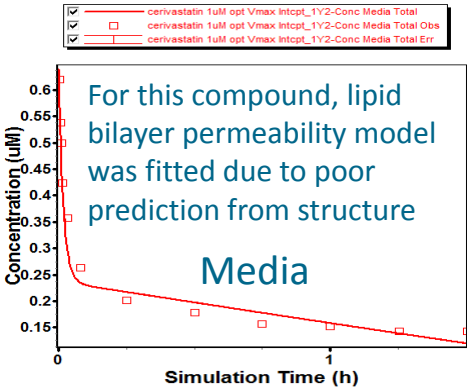


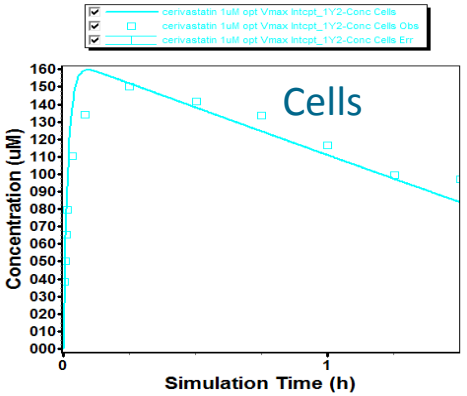




Cerivastatin







MEMBRANEPLUS MODEL RESULTS II

TABLE 2

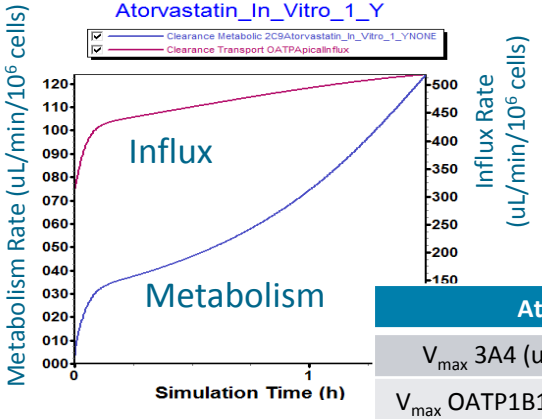
Derived and associated predicted in vitro parameters for atorvastatin, cerivastatin, and indomethacin

Data are mean ± S.D., and n = 3 unless otherwise stated.

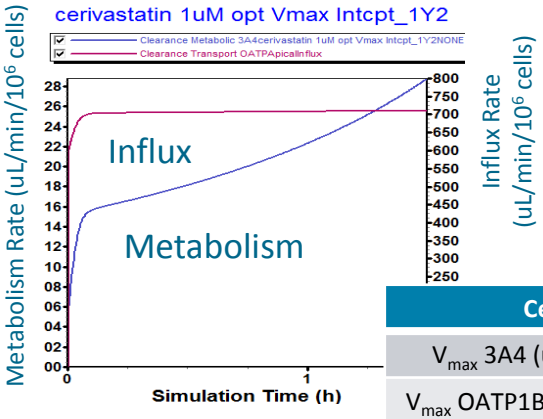
	Atorvastatin	Cerivastatin	Indomethacin
$CL_{int,uptake}$ (μl/min/10^6 cells)	375 ± 45	413 ± 47	599 ± 101
$CL_{int,pass}$ (μl/min/10^6 cells)	17 ± 4.5	58 ± 12	237 ± 63
$CL_{int,met}$ (μl/min/10^6 cells)	4.3 ± 0.65	2.3 ± 0.6	1.0 ± 0.49
Ψ	18	7.8	3.5
k_{mem} (ml)	0.092 ± 0.007	0.15 ± 0.04	0.15 ± 0.13
fu_{cell}	0.011 ± 0.0002	0.0081 ± 0.001	0.054 ± 0.041
CL_{inc} (μl/min/10^6 cells)	10 ± 7	3.0 ± 2.8	4.0 ± 2.3
CL_{med} (μl/min/10^6 cells)	68 ± 31	17 ± 14	7 ± 4
Vss_{med} (ml/10^6 cells)*	8 ± 2	5 ± 1.6	2 ± 0.4
$f_{med,ss}$	0.14 ± 0.04	0.2 ± 0.06	0.54 ± 0.1

* At a cell concentration of 1 × 10^6 cells/ml.

Paine, DMD (2008) 36:1365–1374



Atorvastatin Results	
V_{max} 3A4 (umol/s/L)	0.119
V_{max} OATP1B1(umol/s/L)	31.9
Cl_{met} (uL/min/Mcell)	63 (68 Lit.)
Cl_{upt} (uL/min/Mcell)	471 (375 Lit.)



Cerivastatin Results	
V_{max} 3A4 (umol/s/L)	3.74E-02
V_{max} OATP1B1(umol/s/L)	295.28
Cl_{met} (uL/min/Mcell)	20 (17 Lit.)
Cl_{upt} (uL/min/Mcell)	704 (413 Lit.)

MODEL COMPARISON

- The mechanistic model in MembranePlus achieved similar result as the simpler compartmental model with fewer fitted parameters

	Compartmenal model*	Membrane Plus
Intracellular volume	Fitted	System parameter
Cell membrane volume	Fitted	System parameter
Membrane/water partitioning (kmem)	Fitted	Predicted from compound properties
Active uptake	Fitted	Fitted
Passive diffusion	Fitted	Predicted from compound properties (atorvastatin only)
Metabolism	Fitted	Fitted

Case Studies: *in vitro* to *in vivo* Extrapolation

Case Study 3: Metabolic IVIVE

PROPAFENONE HUMAN HEPATOCYTE DATA

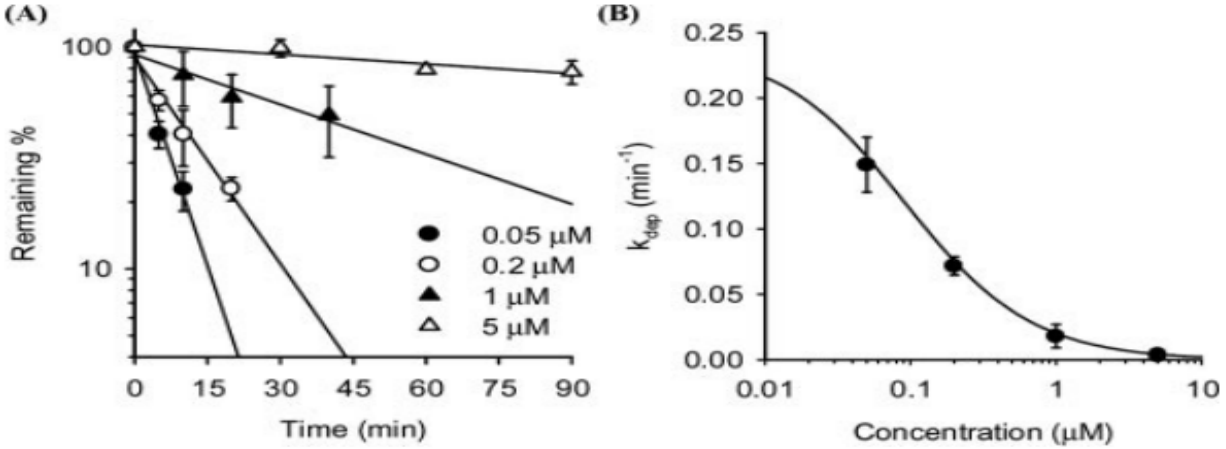
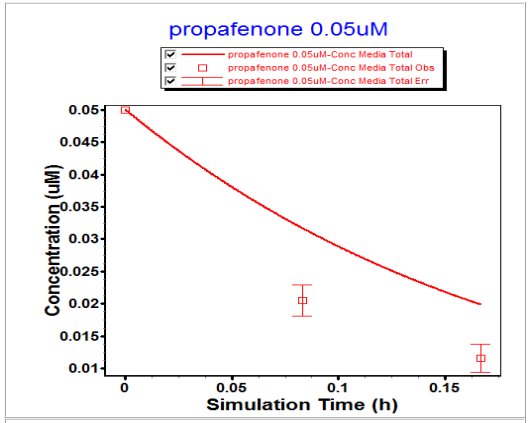


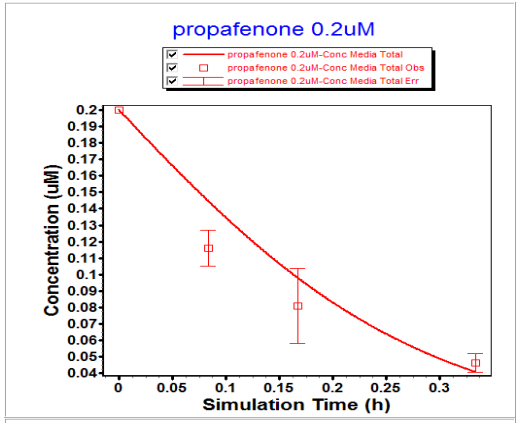
FIG. 2. A, disappearance of propafenone in cryopreserved human hepatocytes at various initial concentrations. Propafenone at concentrations of 0.05, 0.2, 1, and 5 μM was incubated with 1×10^6 cells/ml human hepatocytes individually prepared from three donors. Each point represents the mean ± S.D. of the remaining percentage of substrate to the initial concentration in hepatocytes from three donors. B, relationship between initial substrate concentration and depletion rate constant of propafenone in cryopreserved human hepatocytes. Each point represents the mean ± S.D. of depletion rate constants of three donors. The line represents the curve predicted from eq. 1.

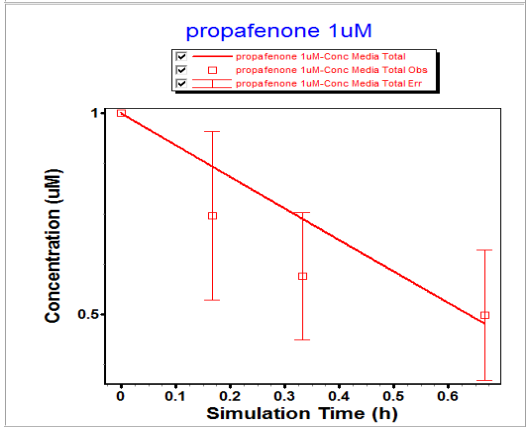
Komura, *Drug metabolism and disposition* 33.6 (2005): 726-732.

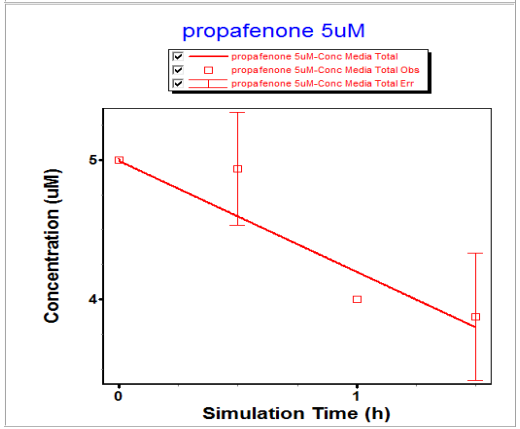
- Reports indicate it is a CYP2D6 substrate and has saturable dose dependent kinetics.
- *In vitro* data from literature was used to fit intracellular unbound Km and Vmax

FIT Km AND Vmax TO *in vitro* DATA









- K_m = 0.0146 mM
- V_{max} = 9.27E-02 mmol/s/L cytosol (Converts to: 2.17E-02 nmol/min/10^6 cells)

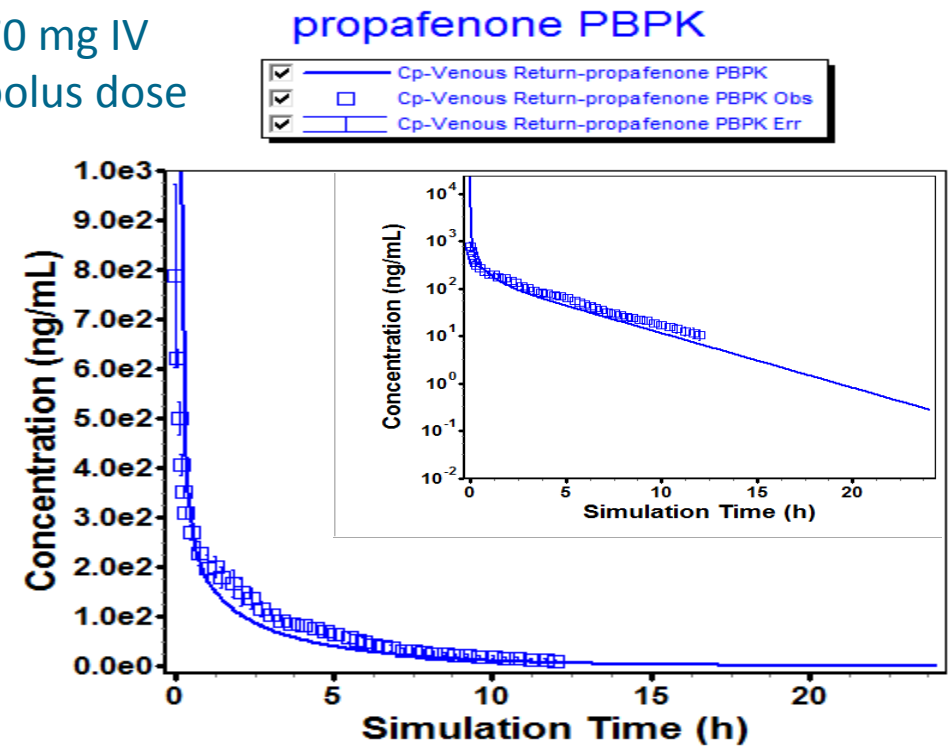
K_m fitted in mechanistic model was lower than the one reported in paper which is indicative of K_m based on unbound intracellular concentration

PREDICT *in vivo* PK

PBPK model:

- Kps predicted using default (Lukacova) method in GastroPlus
- CYP2D6 clearance extrapolated from fitted *in vitro* values (shown on previous slide)

70 mg IV bolus dose

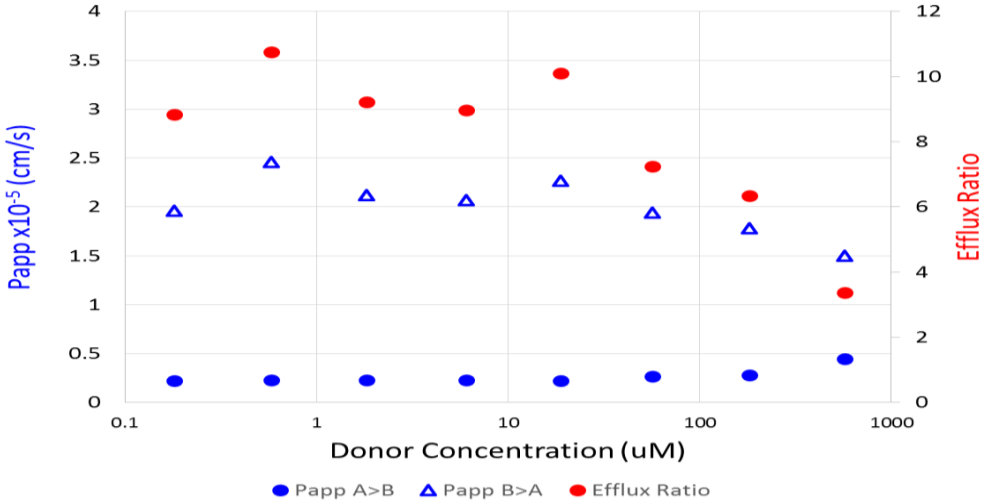


Data: Hollmann, Cardiac Arrhythmias, Springer 125-132

Case Study 4: Transporter IVIVE

DIGOXIN: FIT INTRACELLULAR Km *in vitro*

- Published data on nonlinear Papp vs. donor concentration for Digoxin were used to fit Pgp Km (intracellular unbound) and Vmax



Data from:
Troutman and Thakker, Pharm. Res., Vol. 20, No. 8: 1200-1209.

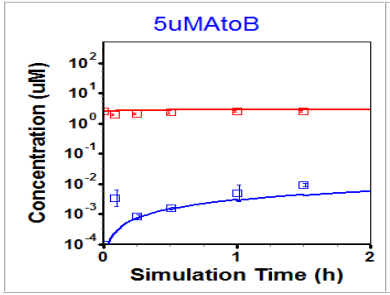
Transporter Table

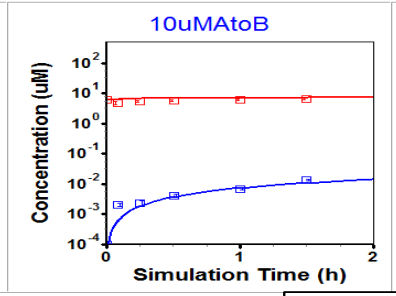
Transporter Table

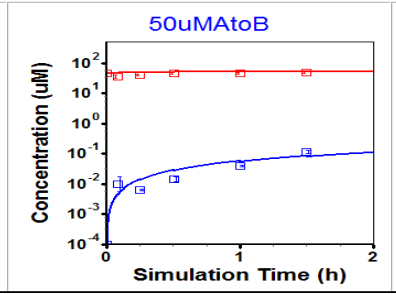
Generic	Transporter	Type	Location	Vmax (umol/s/L Cytosol)	Km (uM)
digoxin_0.18uM_AtoB	P-gp	Efflux	Apical	6.04E+02	9.53E+01

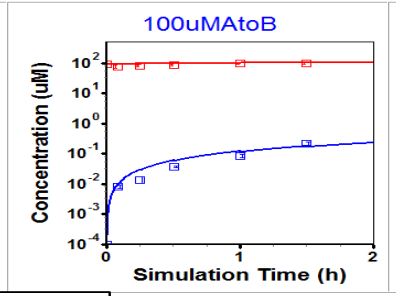
DIGOXIN: MODEL VERIFICATION

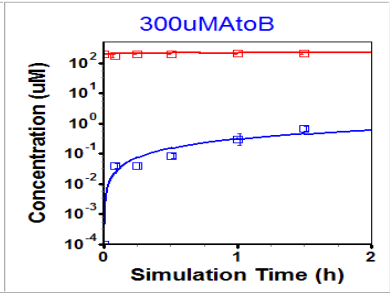
- Km obtained from fitting to a published dataset was used to predict concentration-time profiles from another dataset
- Vmax was adjusted to account for different expression levels of Pgp in different systems

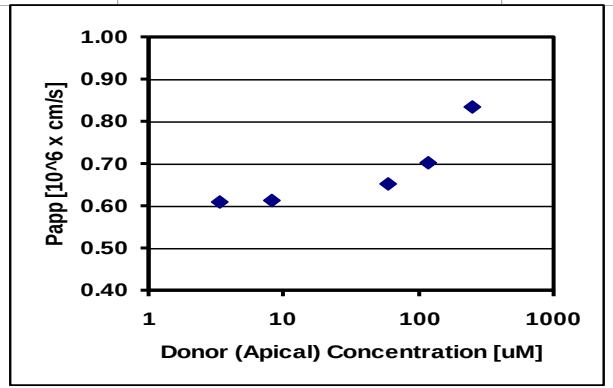




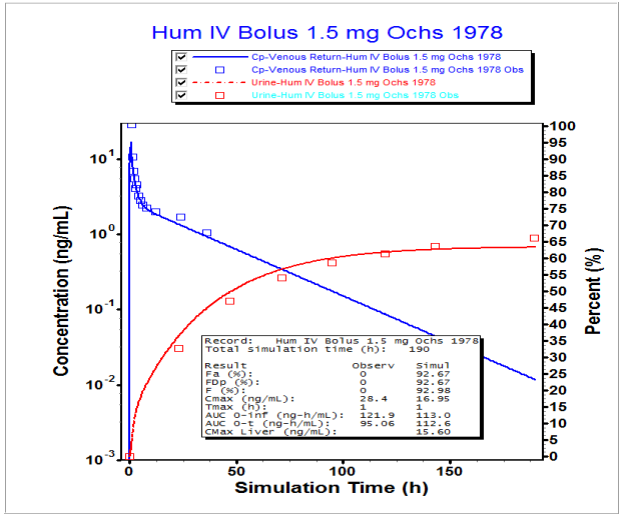




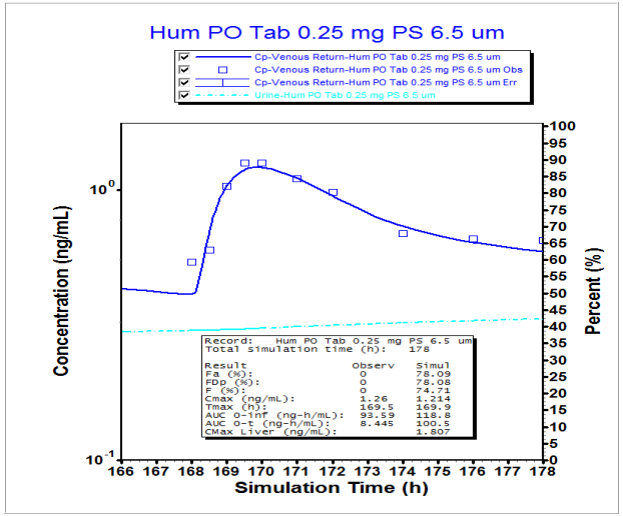




DIGOXIN: PREDICT *in vivo* ABSORPTION



A



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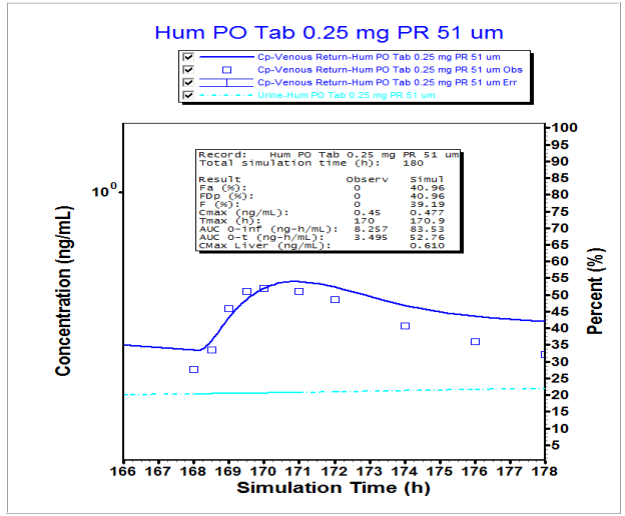


C

A: Observed (symbols) vs. predicted plasma conc. (blue) and urinary excretion (red) of digoxin (Ochs, 1978).

B: Observed (symbols) vs. predicted plasma conc. (blue) of digoxin for a PO formulation with 6.5 mm radius particle size (Jounela, 1975).

C: Observed (symbols) vs. predicted plasma conc. (blue) of digoxin for a PO formulation with 51 mm radius particle size (Jounela, 1975).

All simulations are using the fitted intracellular unbound P-gp Km value of 95.3 mM

Case Studies 5: Lysosomal Trapping and Absorption

LYSOSOMAL TRAPPING OF LIPOPHILIC CATIONS

Lipophilic Amines
LogP > 1
pKa > 6.5

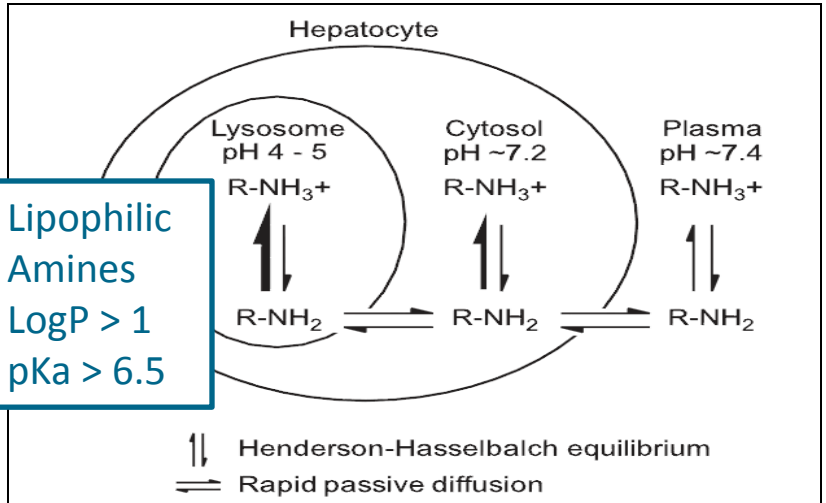
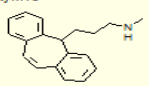
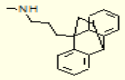
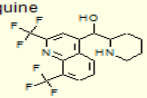
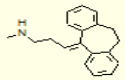
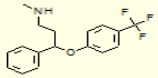
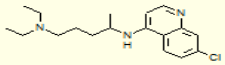


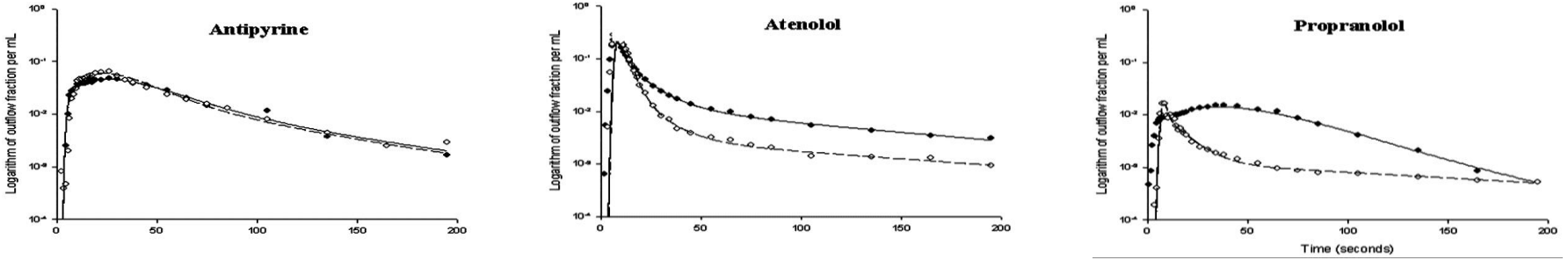
Fig. 1. The basis of pH partitioning of lipophilic amines into lysosomes. The diagram illustrates the mechanism by which lipophilic amines (i.e., CADs) accumulate in lysosomes. From plasma (pH 7.4) and cytosol (~7.2), a lipophilic amine ($\log P > 1$, $pK_a > 6.5$) will readily diffuse across membranes in its unionized form (RNH_2) while maintaining Henderson-Hasselbach equilibrium with its ionized form (RNH_3^+, which cannot readily diffuse across membranes). After diffusion into the acidic environment of the lysosome (pH 4–5), the equilibrium between charged and uncharged species shifts in favor of the ionized form of the lipophilic amine, limiting diffusion of the drug back into the cytosol and, in effect, trapping the drug in lysosomes. For highly permeable lipophilic amines, the concentration of unionized drug (RNH_2) at equilibrium is assumed to be the same in all three compartments (lysosomes, cytosol, and plasma). The figure is not to scale; lysosomes make up about 1% of the hepatocyte volume.

Drug	Log P	Basic pKa	T_{max} (h)
Protriptyline 	4.69	10.0	27
Maprotiline 	4.7	10.1	16
Mefloquine 	3.81	8.52	15
Nortriptyline 	4.46	9.65	7.8
Fluoxetine 	4.39	9.82	7
Chloroquine 	5.11	9.86	6

Kazmi F., Drug Metab. Disp. 41(3):897 (2013)

LYSOSOMAL TRAPPING OF LIPOPHILIC CATIONS

Hepatic perfusion experiment with and without H+ ionophore Monensin



Empty symbols represent controls and solid symbols represent monensin treatment. Dashed and solid lines stand for fitted data in control and treatments, respectively.

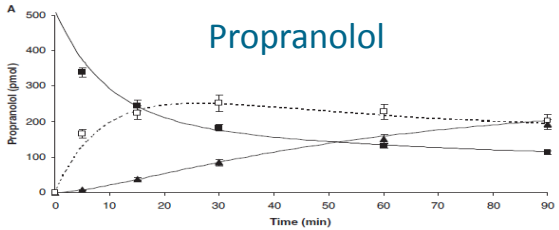
	$\log P_{app}$	pK_a
Atenolol	0.14	9.60
Antipyrine	0.33	1.45
Propranolol	3.10	9.45

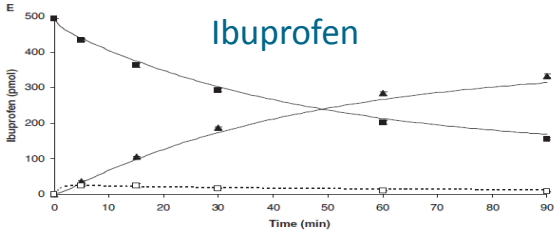
Drug	Monensin Effect
Antipyrine	No Effect
Atenolol	Minor Effect
Propranolol	Strong Effect

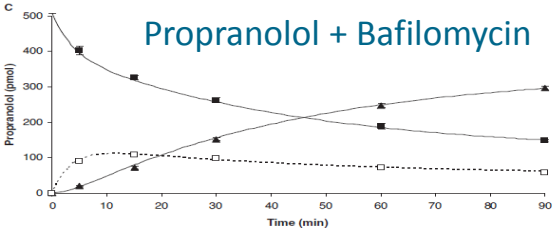
Siebert GA, JPET 308(1):228 (2004))

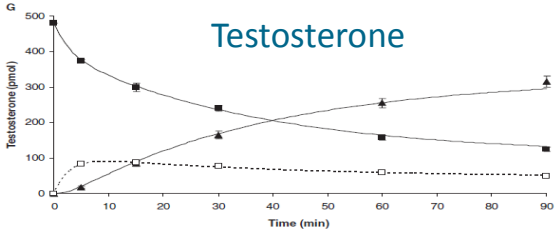
LYSOSOMAL TRAPPING OF LIPOPHILIC CATIONS

Caco-2 Apical-to-Basolateral permeability experiment









Solid squares – Apical compartment
Solid triangles – Basolateral compartment
Empty squares – Cell monolayer
Lines represent fitted model results

Physicochemical properties of the model compounds used

Compound	pK_a[a]	Log D at pH 7.4[a]
Propranolol	9.1_{basic}	1.4
Ibuprofen	4.4_{acidic}	0.8
Testosterone	N.A.[b]	3.5

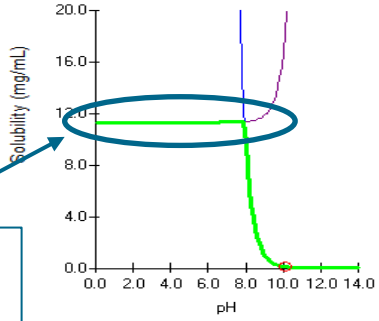
Heikkinen AT, JPET 328: 882 (2009)

CONSEQUENCES *in vivo*: DESIPRAMINE

Desipramine physicochemical properties

Parameter	Value	Reference
Molecular weight (g/mol)	266.4	ADMET Predictor
$\log P_{o/w}$	4.45	[36]
Ionization constant (pKa)	10.32 (Base)	[36]
Solubility [mg/mL]	0.124 (pH = 10.1)	ADMET Predictor
B/P ratio	1.03	ADMET Predictor
Fraction unbound (f_u)	0.19	[36]
Peff (cm/s * 10^4)	4.54 (Human)	[19]

Samant T et al. CPT: PSP, 6(5): 315-321, 2017.

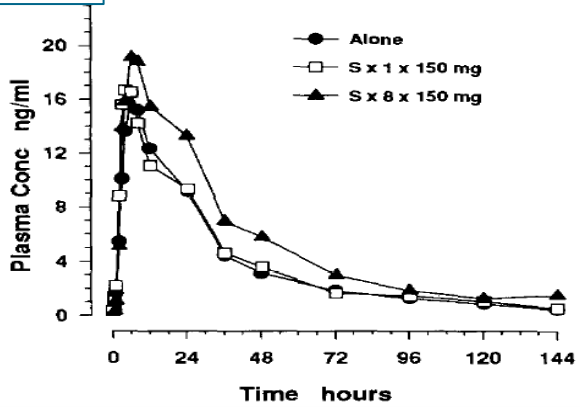


High permeability and intestinal solubility

Late Tmax

Pharmacokinetic parameter	*Given alone (n = 6)*
$t_{1/2}$ (hr)	19.6 ± 7.2
C_{max} (ng/ml)	16.8 ± 6.6
t_{max} (hr)	6.7 ± 1.6
AUC(0-∞) (ng · hr/ml)	516 ± 266
CL/F (L/hr)	118 ± 53.5
V/F (L)	3110 ± 1490

Kurtz D.L. et al. CPT 1997, 62: 145-156

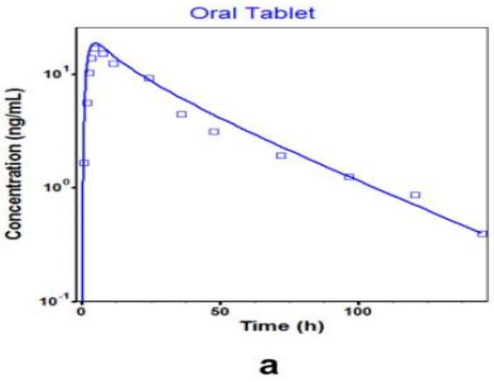


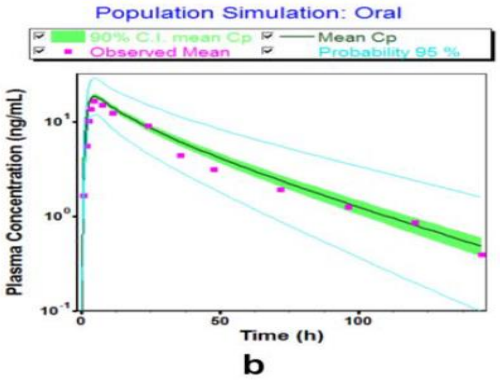
CONSEQUENCES *in vivo*: DESIPRAMINE

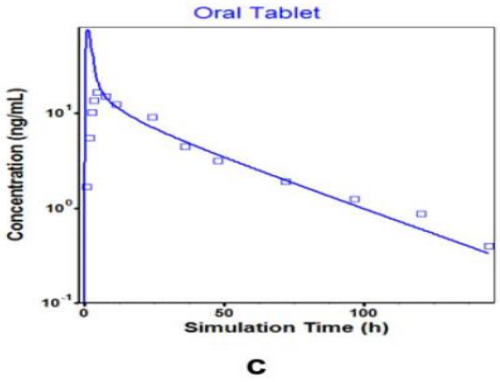
Fu ent = 0.55%

Fu ent = 100%

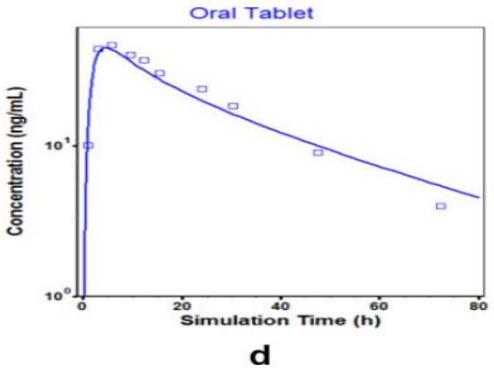
50 mg dose

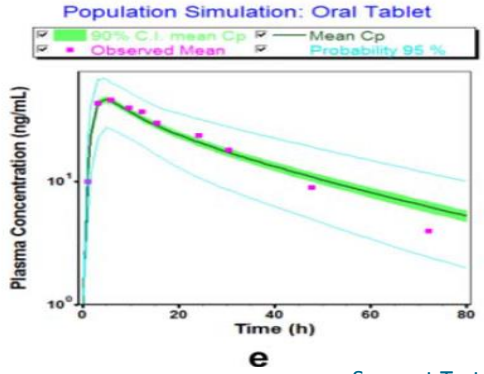






100 mg dose





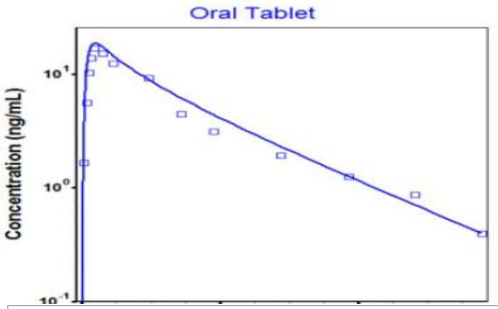
Samant T et al. CPT: PSP, 6(5): 315-321, 2017.

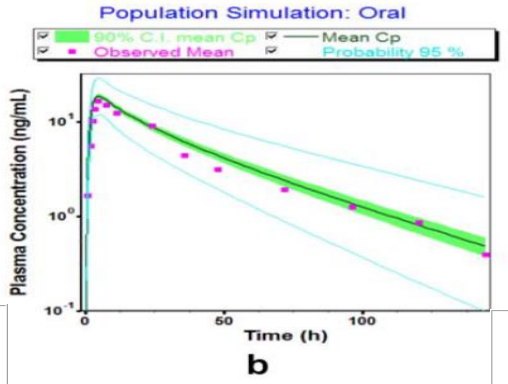
CONSEQUENCES *in vivo*: DESIPRAMINE

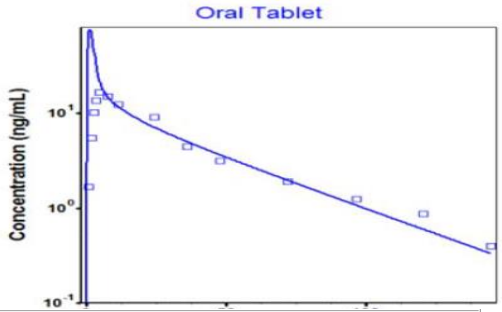
Fu ent = 0.55%

Fu ent = 100%

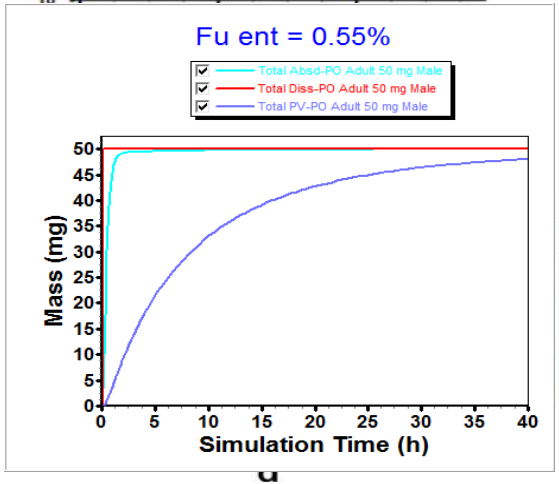
50 mg dose

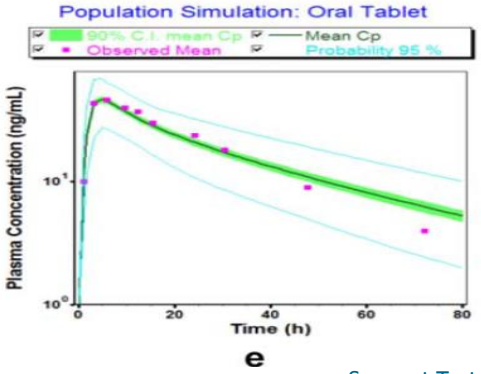


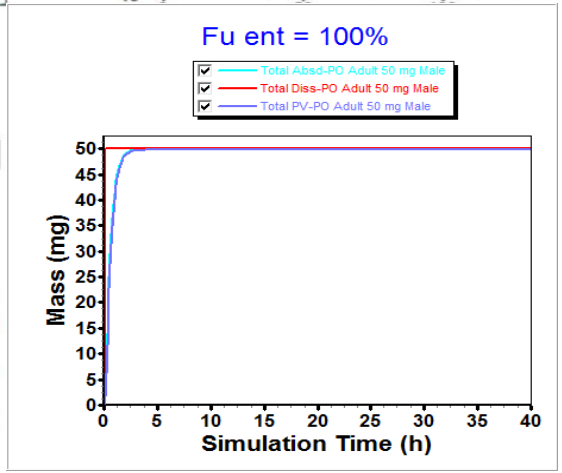




100 mg dose

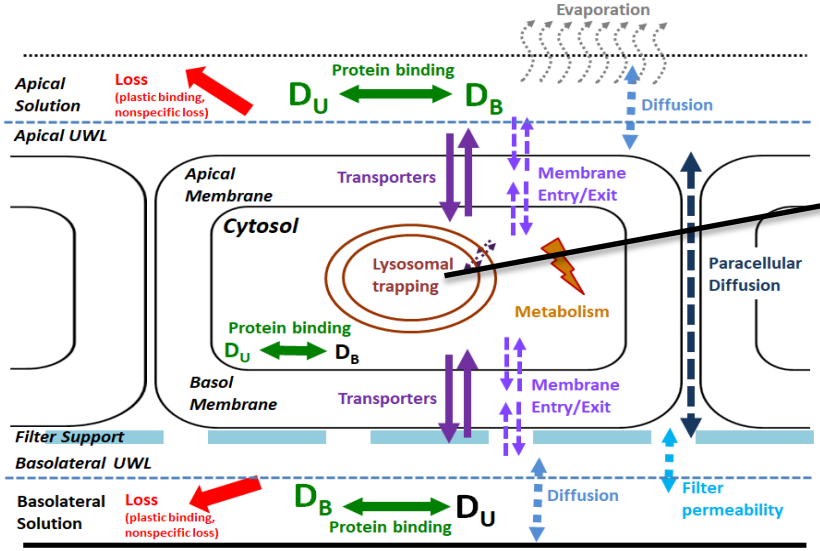


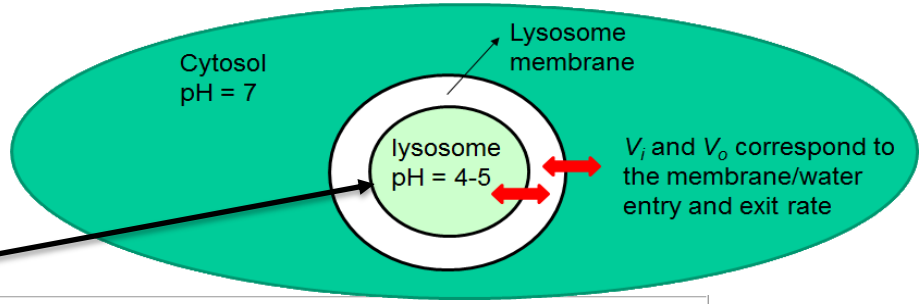




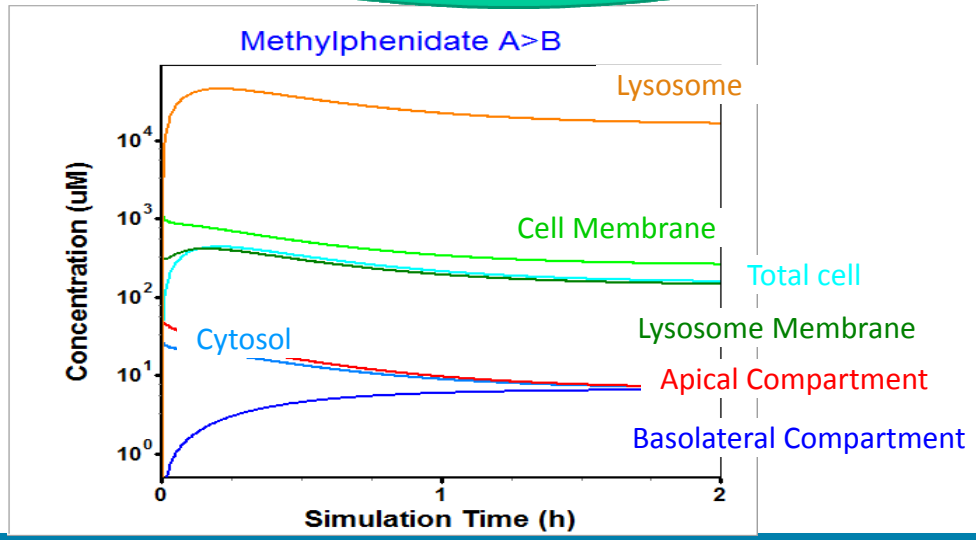
Samant T et al. CPT: PSP, 6(5): 315-321, 2017.

METHYLPHENIDATE MEMBRANEPLUS™ SIMULATION

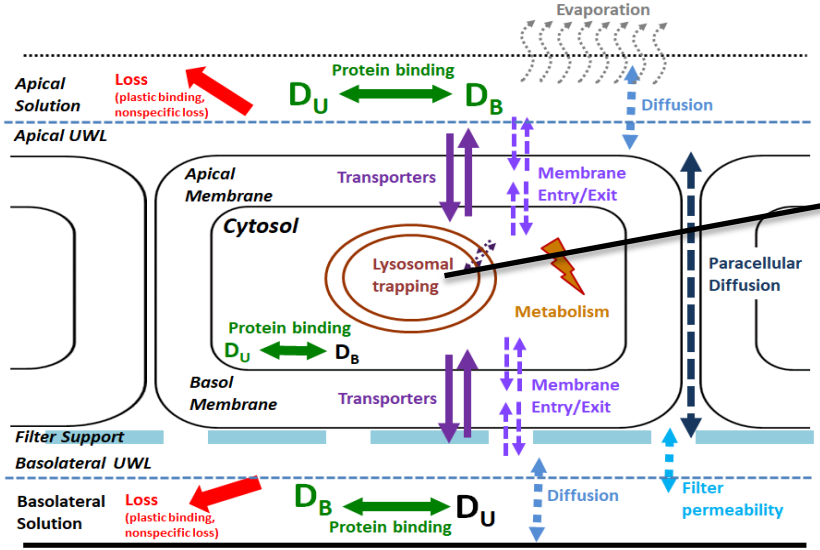


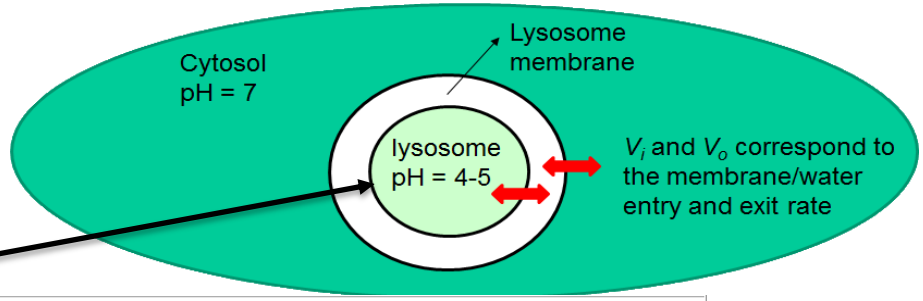


S+LogP = 2.02 (AP 7.2)
S+pKa = 8.56 (Base) (AP 7.2)

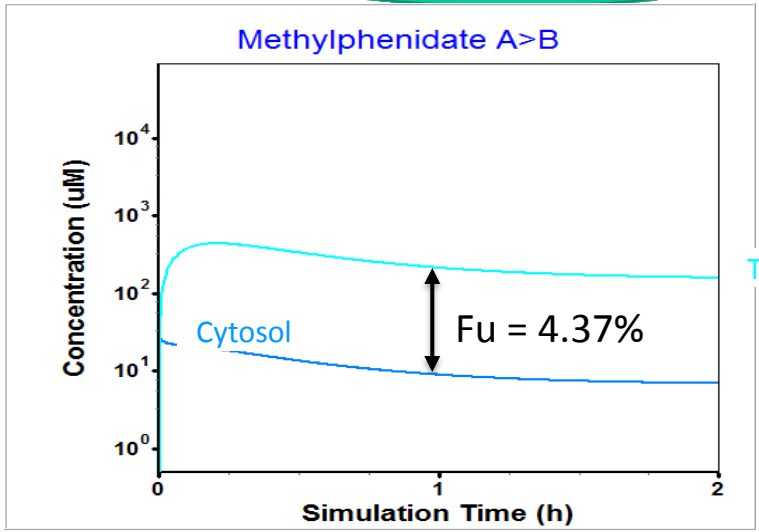


METHYLPHENIDATE MEMBRANEPLUS™ SIMULATION



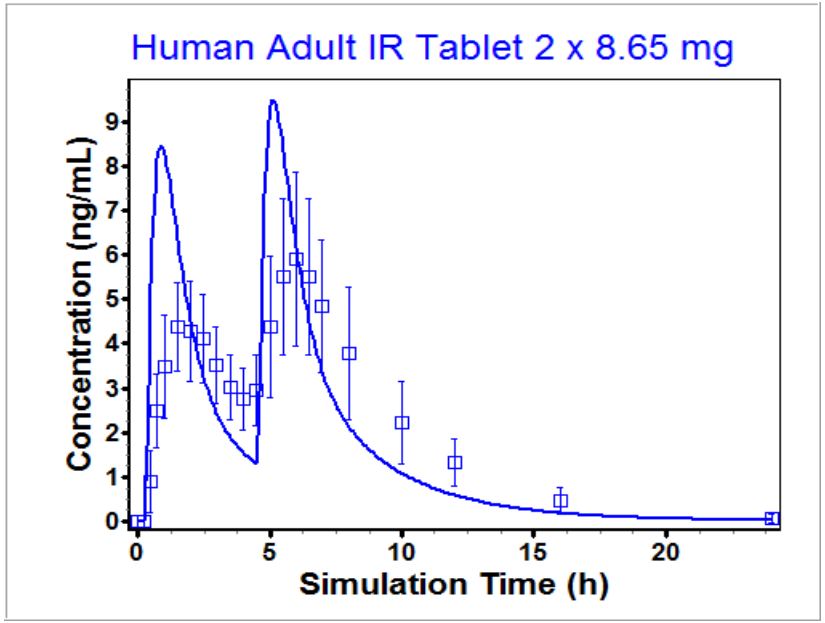


S+LogP = 2.02 (AP 7.2)
S+pKa = 8.56 (Base) (AP 7.2)

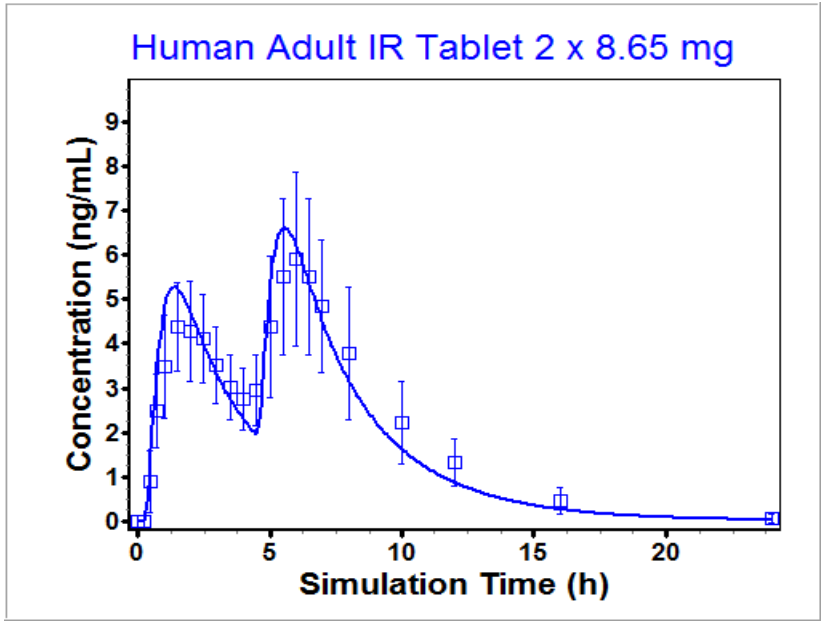


METHYLPHENIDATE GASTROPLUS SIMULATION

Fu,ent = 100%



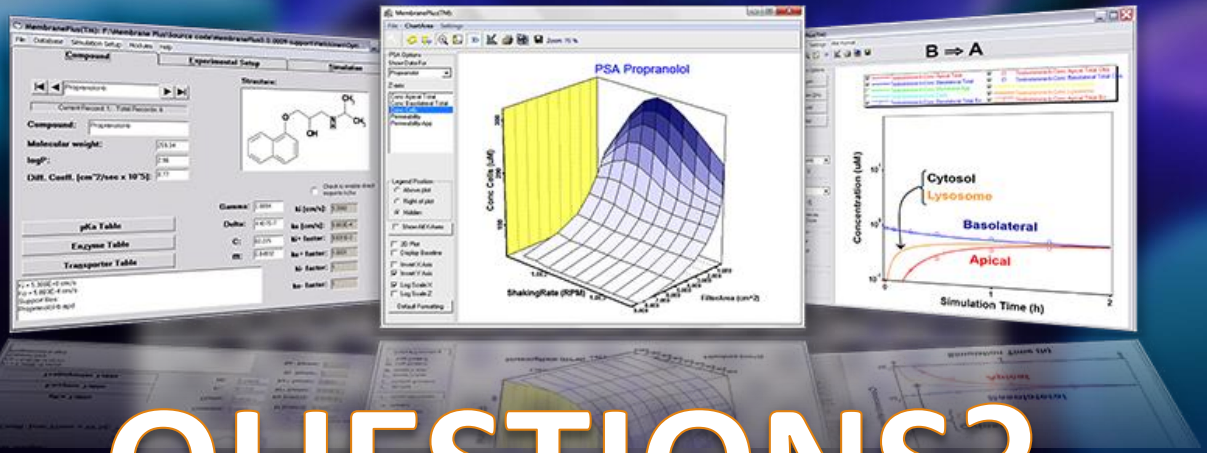
Fu,ent = 4.37%



SUMMARY

- Mechanistic cellular simulations:
  - Help to separate drug and system parameter inputs (similar to *in vivo* PBPK models) for easier translation between systems
  - Are important to assess critical mechanisms affecting drug absorption, distribution and elimination pathways:
    - Contributions of passive and active drug transport
    - Interplay between drug metabolism and cellular uptake
    - Disposition in bile
    - Distribution within cellular structures (lipid bilayers, lysosomes)
  - Facilitate extraction of variety of relevant parameters for more accurate IVIVE

MembranePlus™



QUESTIONS?

SimulationsPlus

SCIENCE + SOFTWARE = SUCCESS

22ND NORTH AMERICAN ISSX MEETING

Additional Slides

COMPOUND X - CLASS II/IV

AAPS PharmSciTech (© 2014)
DOI: 10.1208/s12249-014-0075-1

Research Article

Application of Physiologically Based Absorption Modeling to Formulation Development of a Low Solubility, Low Permeability Weak Base: Mechanistic Investigation of Food Effect

Hefei Zhang,[1,2] Binfeng Xia,[1] Jennifer Sheng,[1] Tycho Heimbach,[1] Tsu-Han Lin,[1] Handan He,[1] Yanfeng Wang,[1] Steven Novick,[1] and Ann Comfort[1]

Received 2 October 2013; accepted 23 December 2013

Table II. Physicochemical Parameters, Default Physiological Values, and Pharmacokinetic Parameter Used in the Simulation at Various Doses

Parameters	Value(s)
Compound parameters	
M_w: g/mol	>475
cLogP:	>4
pK_a (base):	3.2, 6.2
Dosage:	IR capsule
Solubility (mg/mL):	1.8 (pH 1), 0.3 (pH 2), 0.001 (pH 6.8)
Biorelevant solubility (mg/mL) :	0.023 (fasted); 0.190 (fed)
Mean precipitation time (s) :	450 s (fasted); 2,000 s (fed
Particle radius of API (μm):	19
Physiological parameters	
Stomach pH	1.2 (Fasted); 1.2–4.9 (Fed)
Duodenum/jejunum pH	6.0–6.4 (Fasted); 5.4–6.0 (Fed
Ileum pH	6.6–7.4 (Fasted); 6.6–7.4 (Fed
Stomach transit time (h)	2.0 (Fasted); 5.4 (Fed)
Small intestine transit time (h)	3.3
Cecum transit time (h)	4.2
Ascending colon transit time (h)	12.6
Pharmacokinetics	
First pass extraction (%):	9.0
Blood/plasma ratio:	0.68
Plasma unbound (%):	1.6
Clearance (L/h/kg)	0.070
V_c (L/kg)	0.4
k_{12} (1/h)	0.64
k_{21} (1/h)	0.17
V_t (L/kg)	1.5

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- Lipophilic (log P > 4)
- Moderate base (pKa 3.2 and 6.2)
- Low (0.001 mg/mL), pH dependent solubility
- Moderate gut permeability (1.48 x 10^{-4} cm/s)
- Estimated bioavailability ~30%

Are the different (fitted) precipitation and gastric emptying times under fasted & fed conditions masking something else in the model?

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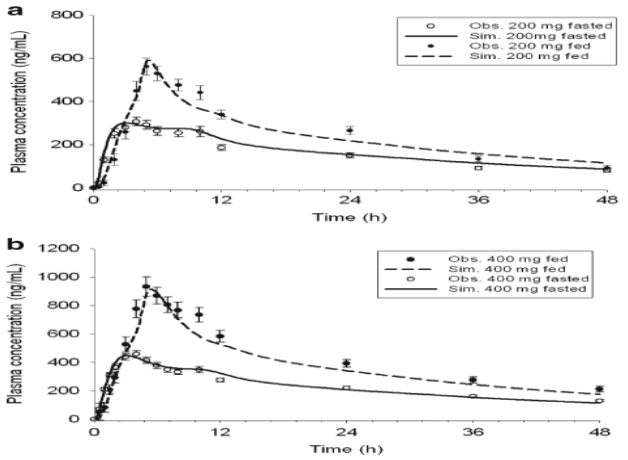
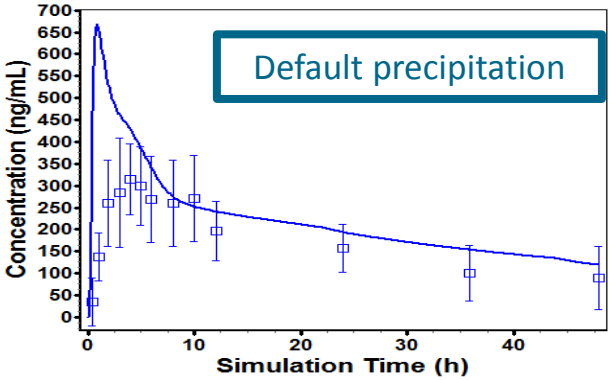
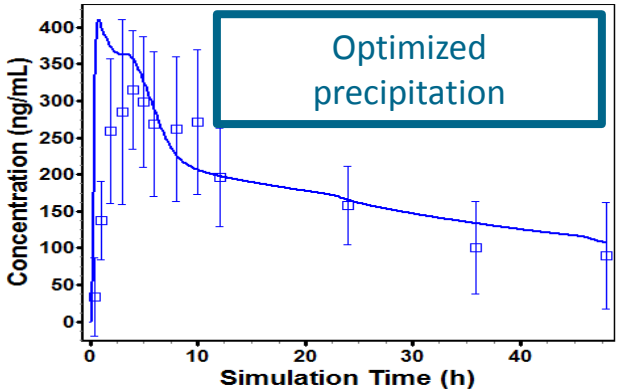


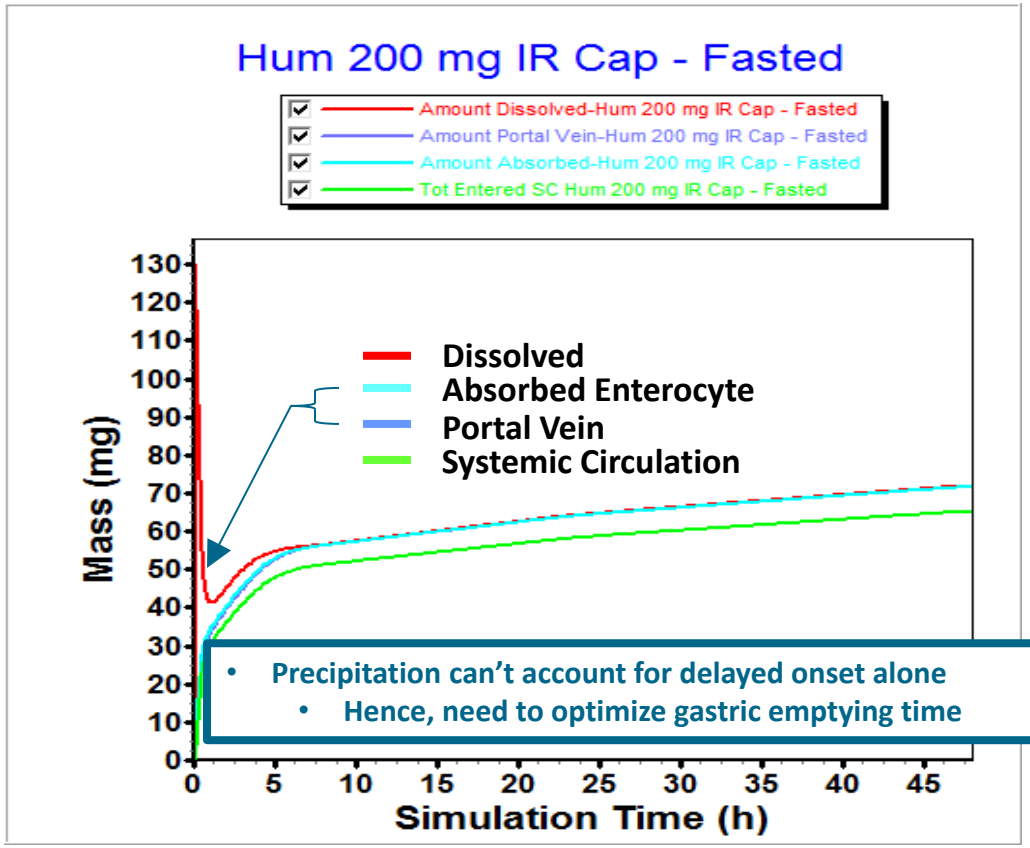
Fig. 1. Mean clinically observed (*solid circles* with standard error) and model-simulated plasma concentration *versus* time profiles of cpd X after a single oral dose of **a** 200 mg or **b** 400 mg cpd X under fasted and fed condition

COMPOUND 'X' – FASTED STATE MODEL DEVELOPMENT

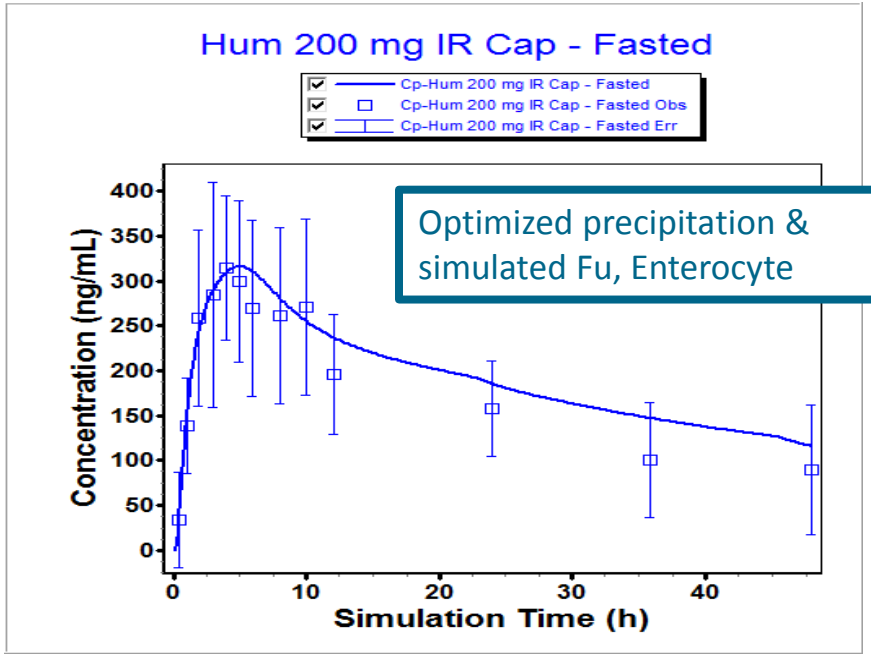
Measured & ACAT™ Default Model Parameters

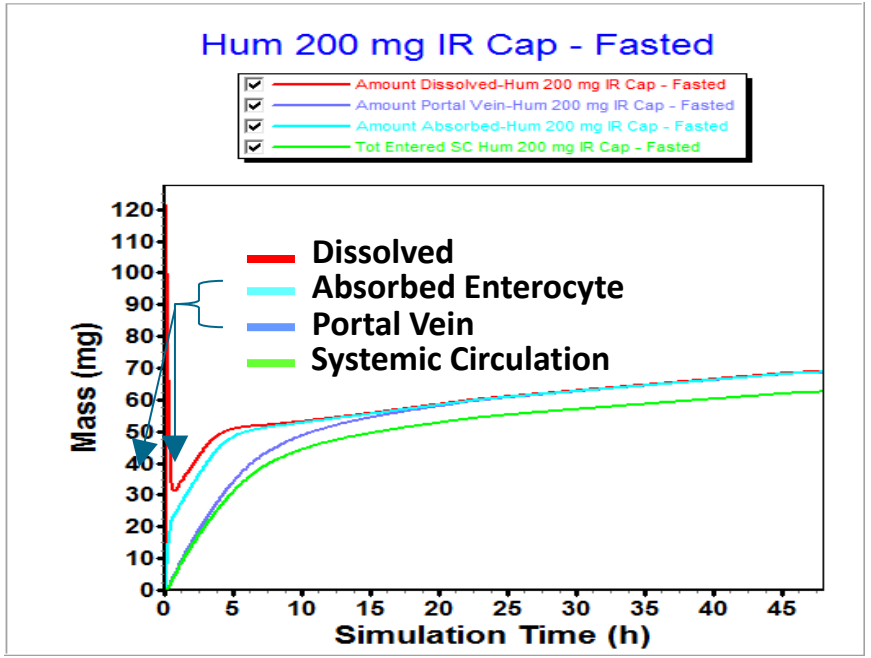






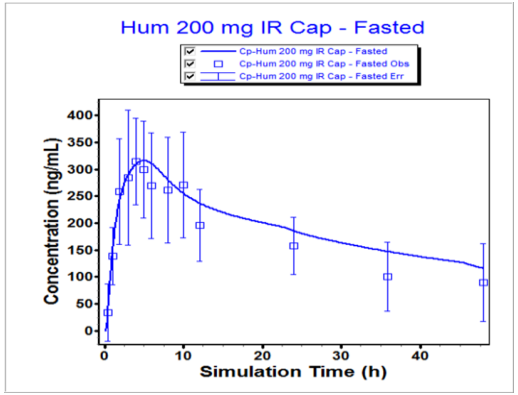
COMPOUND 'X': GASTROPLUS™ SIMULATIONS WITH MEMBRANEPLUS™ fu, ENTEROCYTE = 3.47%

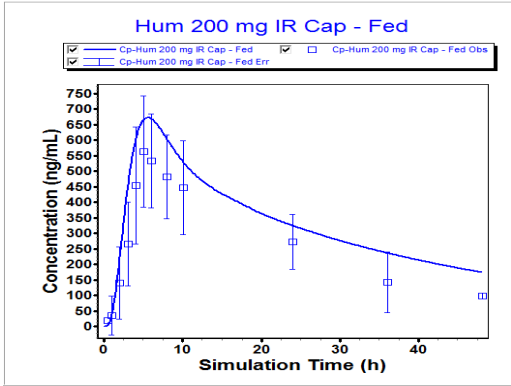


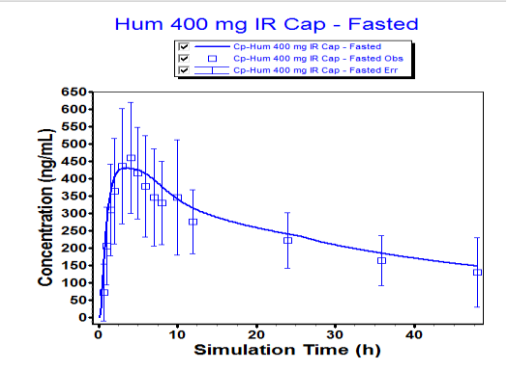


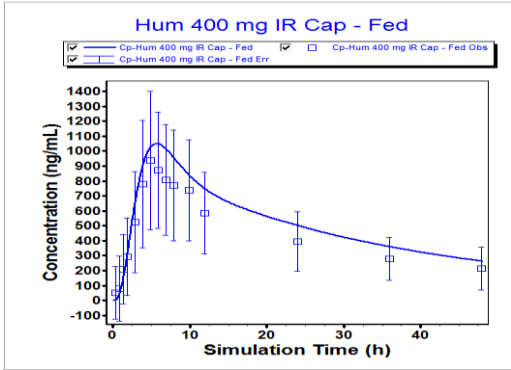
- The lag between absorption into enterocyte and basolateral clearance into portal vein captures the extended Tmax
- **No changes to default GI physiology required**

COMPOUND 'X' – FOOD EFFECT PREDICTIONS ACROSS DOSES









- simulated MembranePlus™ Fu, Enterocyte input
- Optimized precipitation from **low dose/fasted state PK data** >> predicted remaining 3 study arms
- Default ACAT™ fasted/fed physiology parameters

MEMBRANEPLUS

Data Analysis

- Instant permeability output from molecular structure or experimental data.
- Unbound Intracellular concentrations (Membrane, cytosol, lysosome, etc.)
- *In vitro* K_m and V_{max} for enzymes and transporters
- Parameter sensitivity analysis (on logP, shaking rate, pH etc.)
- Transwell, sandwich hepatocyte, and suspended hepatocyte models

Assay Prediction

- Permeability
- Concentrations in cellular structures (Membrane, cytosol, lysosome)