Modeling of Active Transport and Metabolism for *in vitro* Suspended and Sandwich Hepatocyte Assays Utilizing MembranePlus™

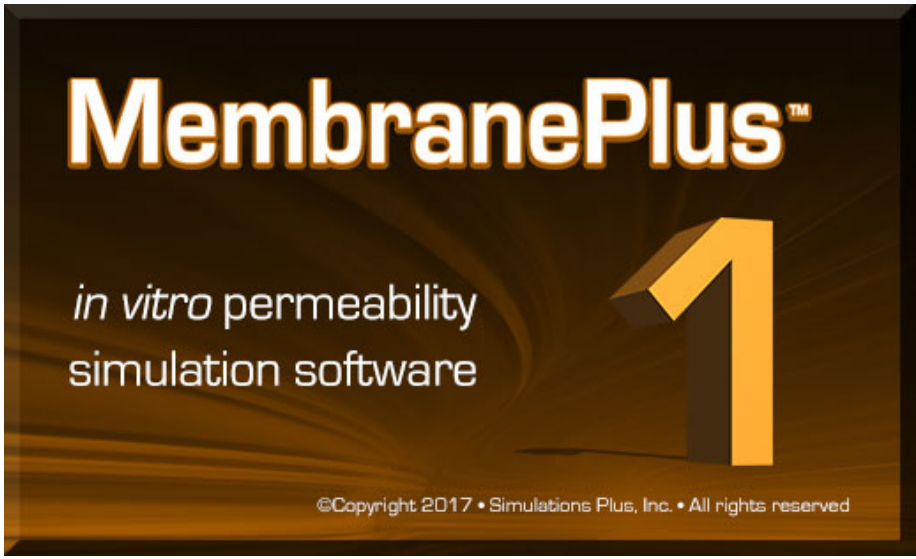
James Mullin
Principal Scientist
Simulations Plus, Inc.

SimulationsPlus
SCIENCE + SOFTWARE = SUCCESS

Overview

- MembranePlus™ – a software platform for simulation of drug transport in cell assays.

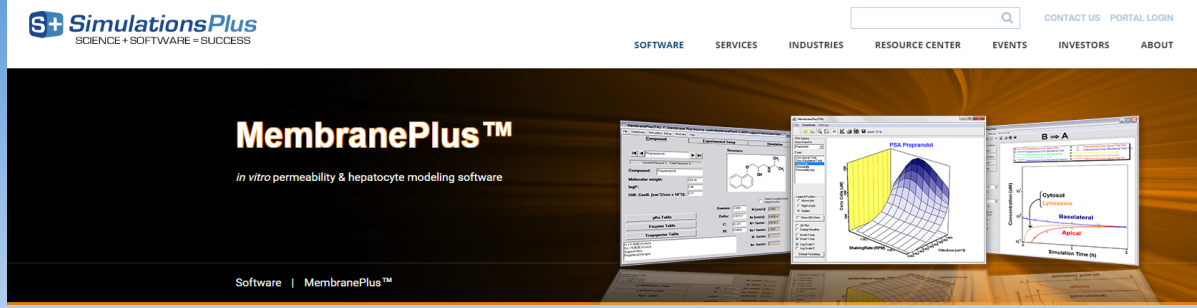
- Development of Hepatocyte Transport Model
  - Sandwich and plated hepatocytes.
  - Suspended hepatocytes.



- Model Case Studies
  - Sodium Taurocholate transport
  - Statins (uptake+metabolism)
  - IVIVE with combination of MembranePlus and GastroPlus

SimulationsPlus
SCIENCE + SOFTWARE = SUCCESS

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.)
- Sandwich and suspended hepatocyte model (version 2 imminent)
- DDI predictions (Coming Soon in Version 3)

Assay Prediction

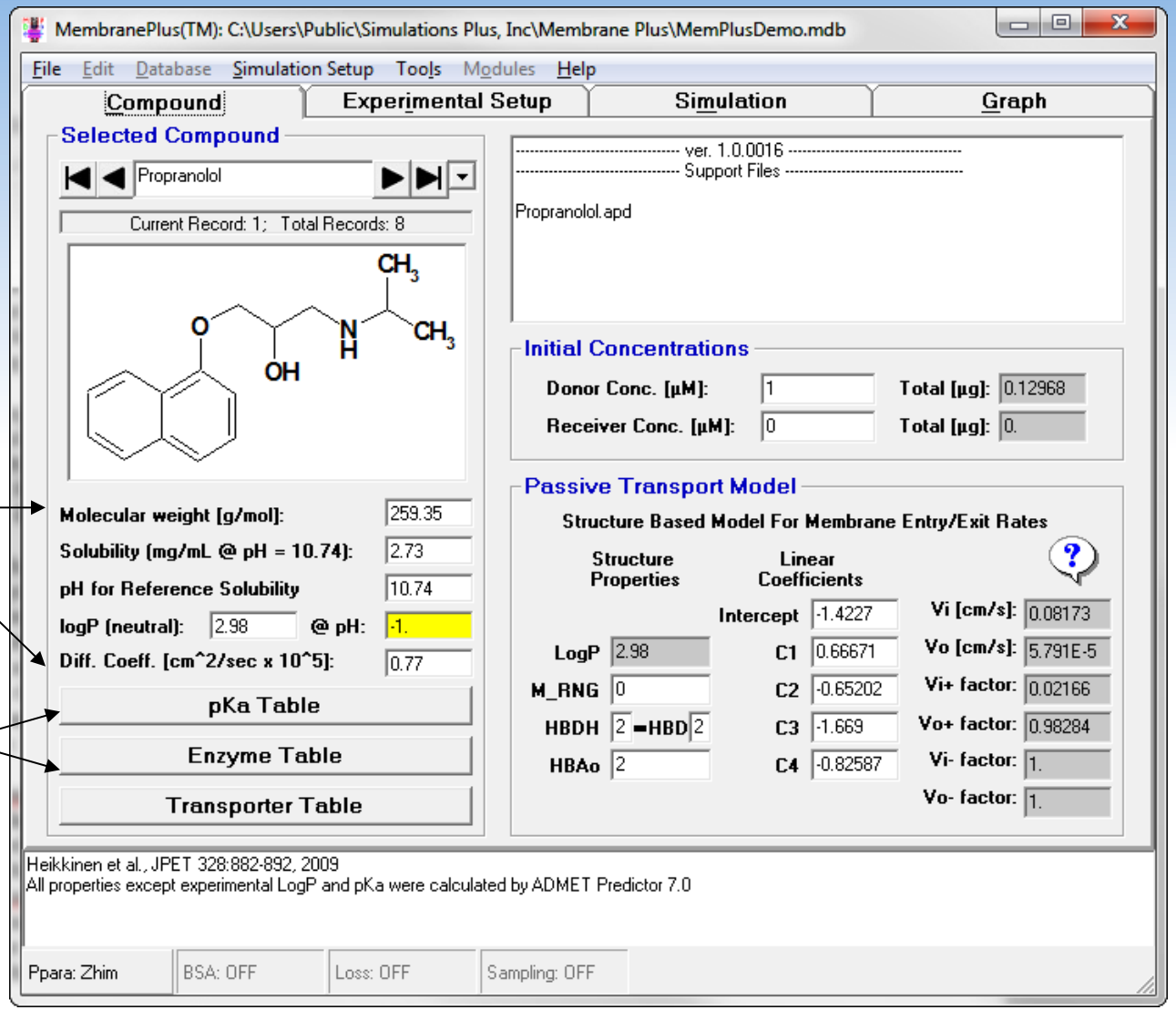
- Permeability
- Intracellular concentrations (Membrane, cytosol, lysosome)
- Virtual Trials (coming soon)

SimulationsPlus
SCIENCE + SOFTWARE = SUCCESS

MembranePlus – Software Overview

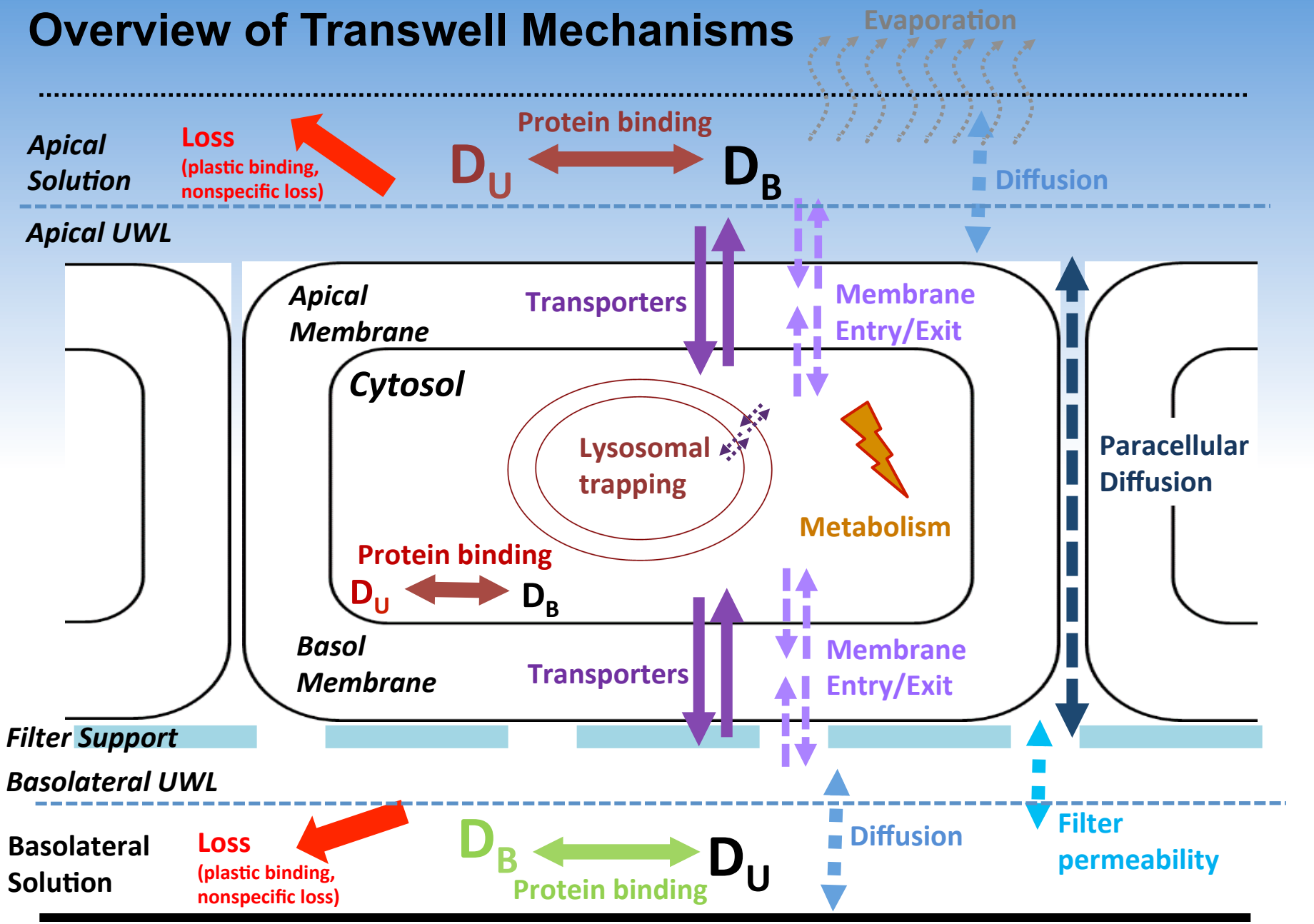
ADMET Predictor™ Values

Applicable only to cell-based assays



SimulationsPlus
SCIENCE + SOFTWARE = SUCCESS

Overview of Transwell Mechanisms

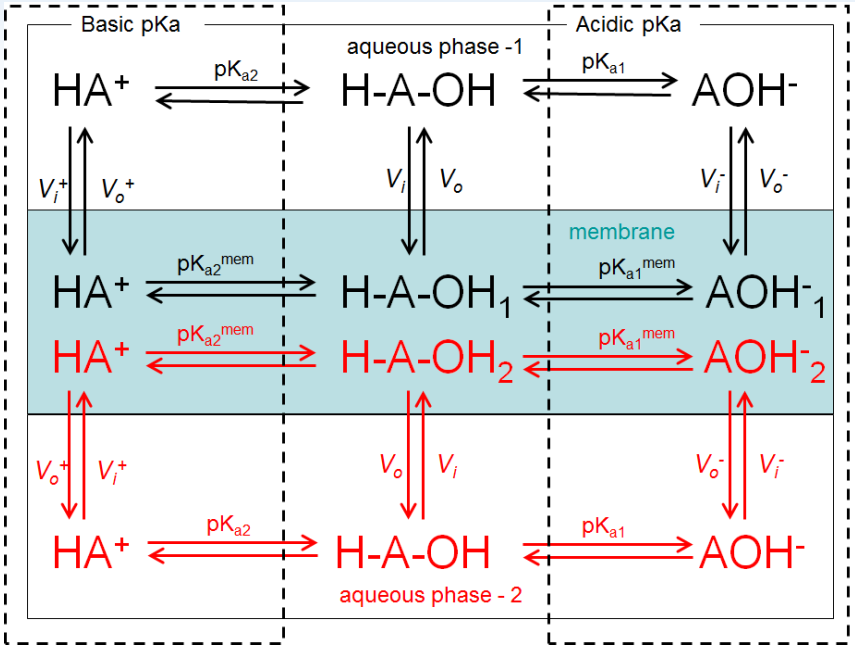


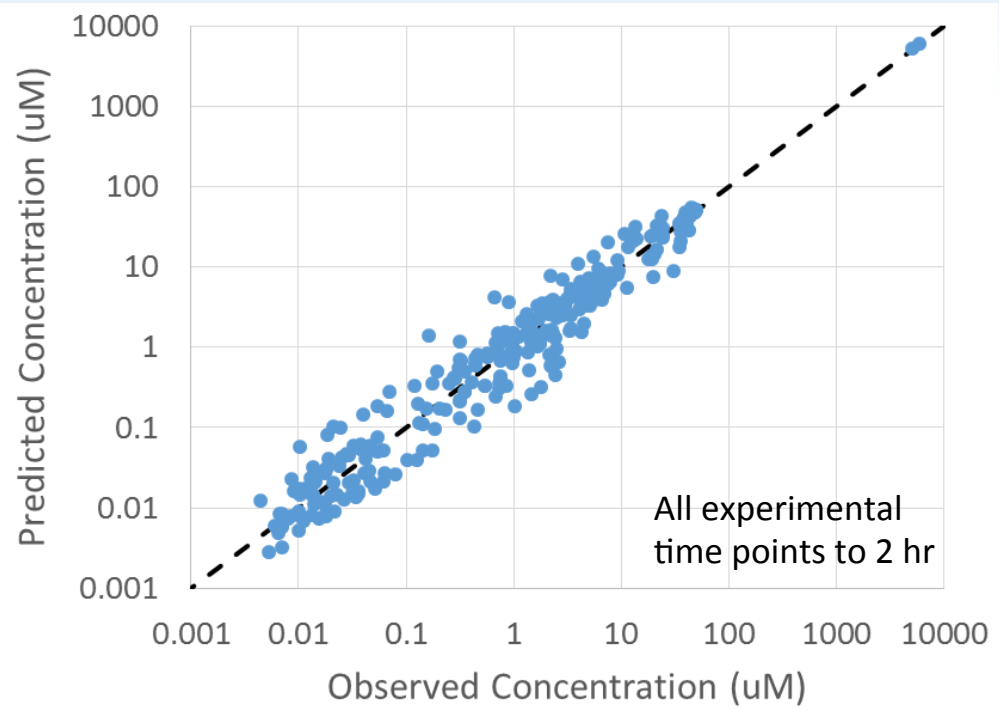
SimulationsPlus
SCIENCE + SOFTWARE = SUCCESS

MembranePlus – 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

SimulationsPlus
SCIENCE + SOFTWARE = SUCCESS

MembranePlus – Lysosomal Trapping Model

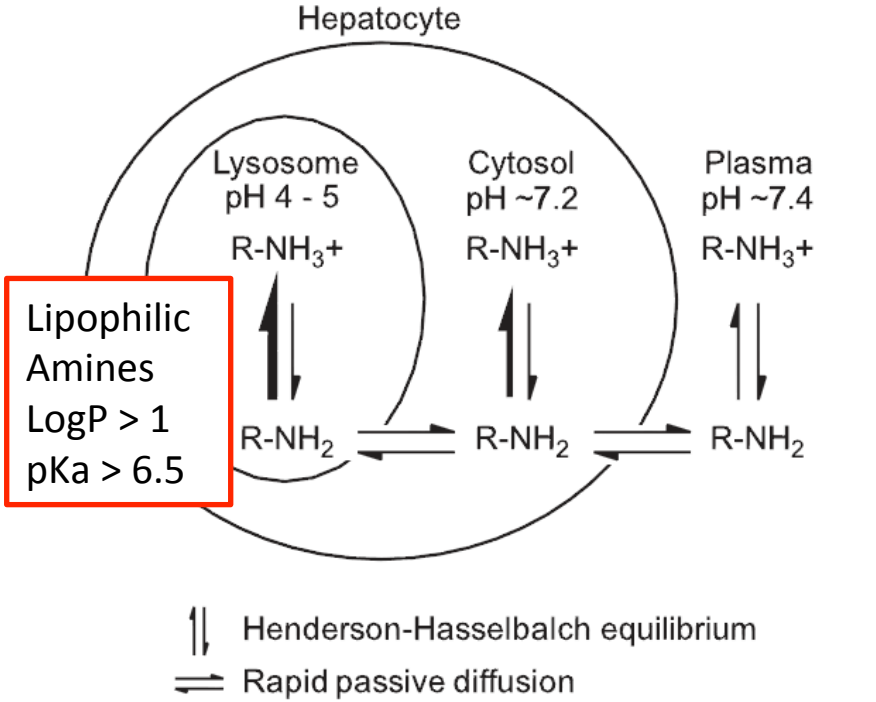
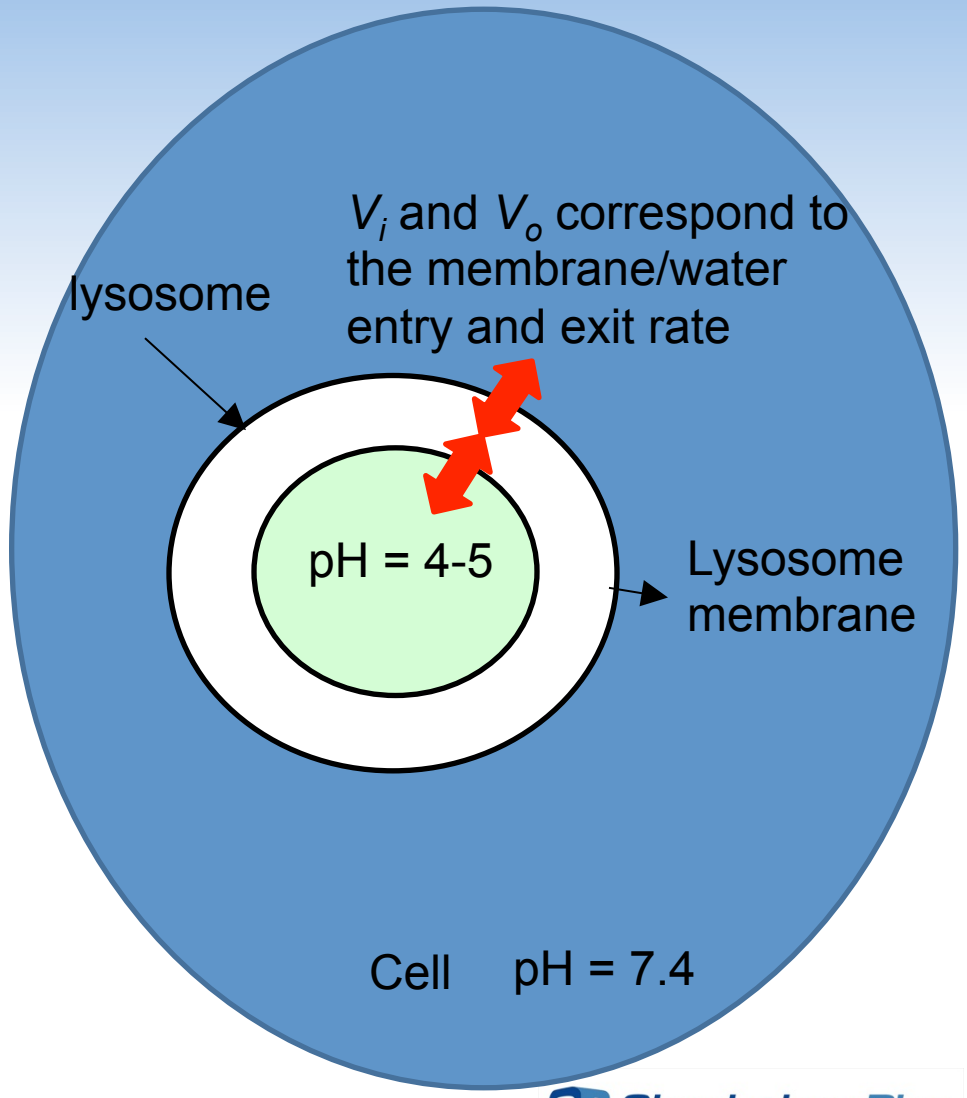


Fig. 1. The basis of pH partitioning of lipophilic amines into lysosomes. The diagram illustrates the mechanism by which lipophilic amines (i.e., CADs) accumulate in lysosomes. From plasma (pH 7.4) and cytosol (~7.2), a lipophilic amine ($\log P > 1$, $pK_a > 6.5$) will readily diffuse across membranes 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.



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

SimulationsPlus
SCIENCE + SOFTWARE = SUCCESS

Enzymes and Transporters - Equations

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

$$\upsilon_{metab} = \sum_i \left(\frac{V_{\max}^i \times c_u^{\text{intracell}}}{K_m^i + c_u^{\text{intracell}}} \right) \qquad \upsilon_{efflux} = \sum_i \left(\frac{V_{\max}^i \times c_u^{\text{intracell}}}{K_m^i + c_u^{\text{intracell}}} \right)$$

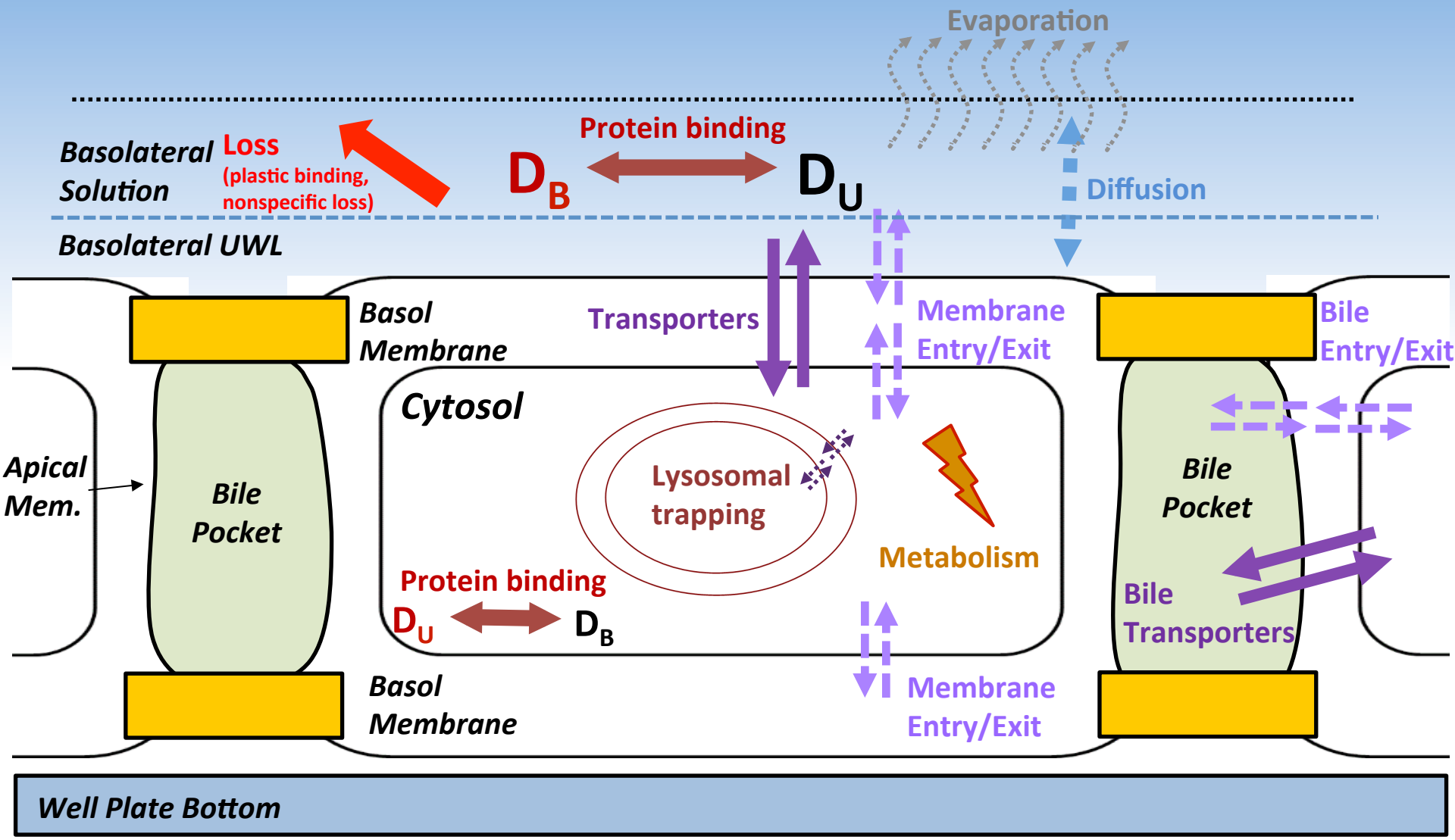
$$\upsilon_{influx} = \sum_i \left(\frac{V_{\max}^i \times c_u^{buffer}}{K_m^i + c_u^{buffer}} \right)$$

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

Sandwich and Suspended Hepatocyte Model

- New to version 2.0 (expected May/June 2017)
- Model non-linear drug uptake and metabolism in:
  - Sandwich Hepatocytes
  - Plated Hepatocytes
  - Suspended Hepatocytes
- Inclusion of intracellular protein binding (free fraction)

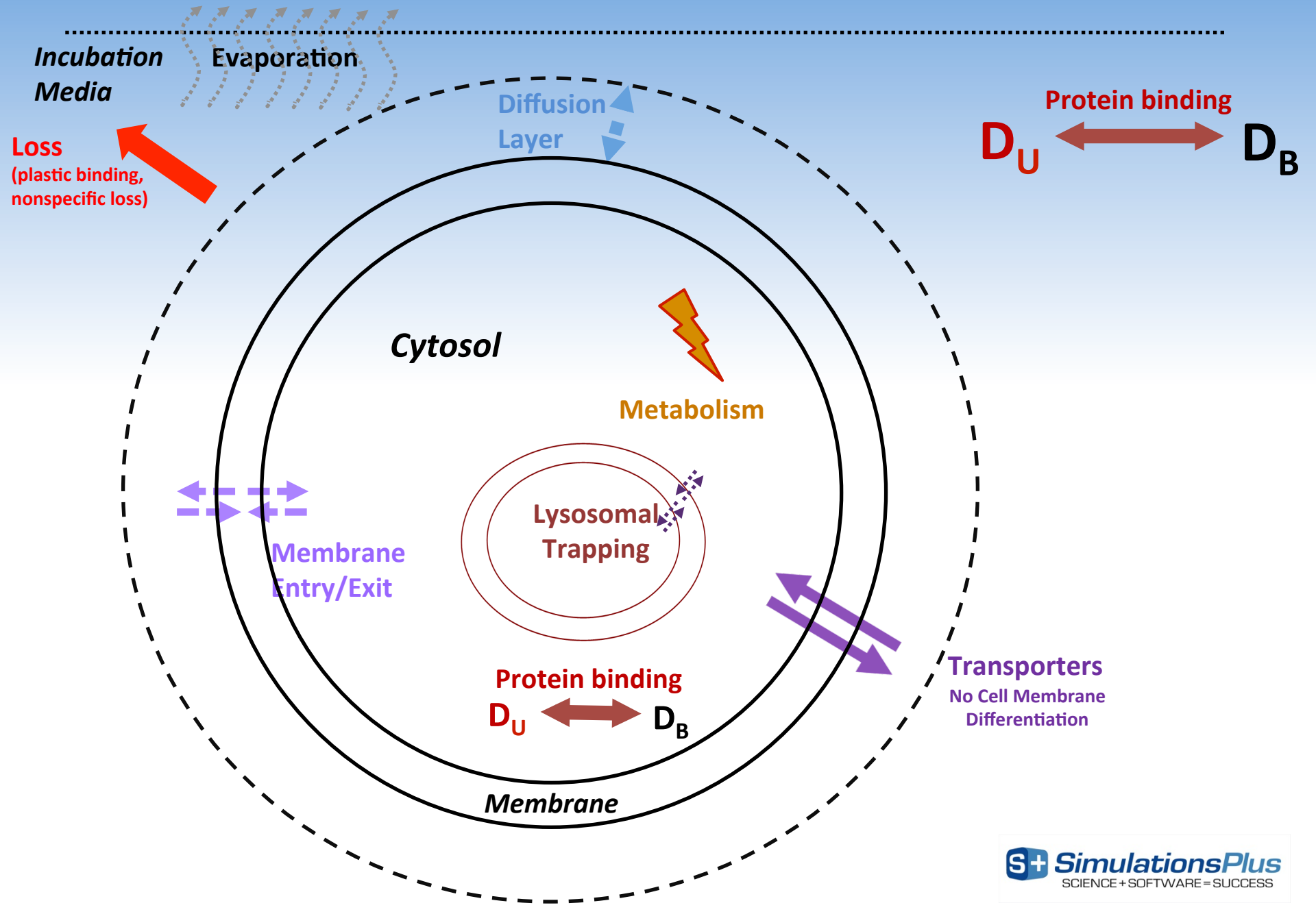
Overview of Sandwich Hepatocyte Mechanisms



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

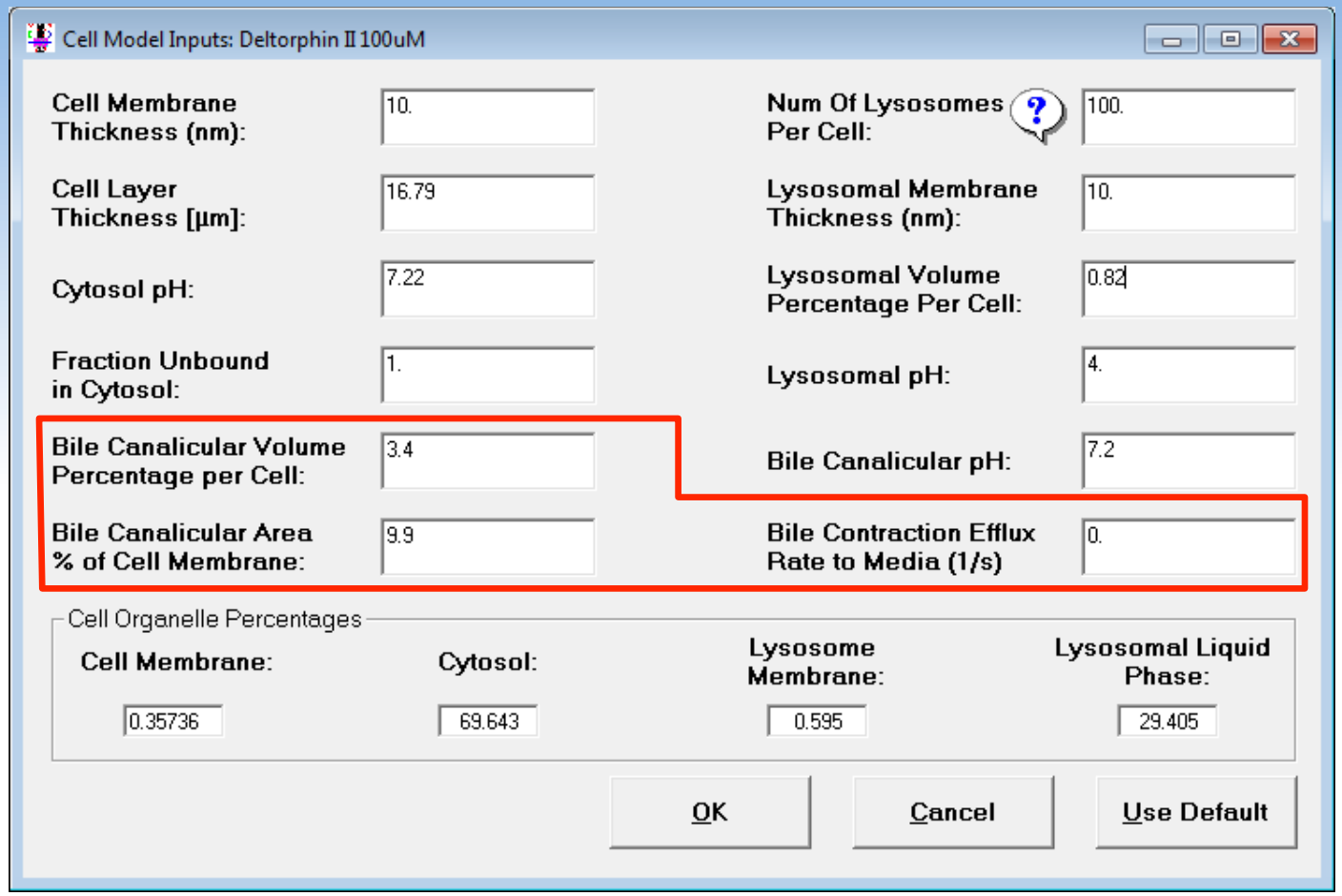
SimulationsPlus
SCIENCE + SOFTWARE = SUCCESS

Overview of Suspended Hepatocyte Mechanisms



SimulationsPlus
SCIENCE + SOFTWARE = SUCCESS

Physiological Settings



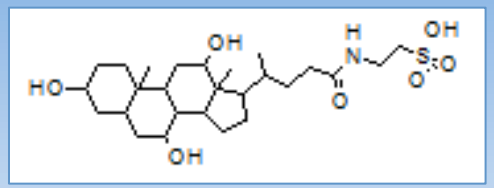
- Plated hepatocytes can be modeled by setting bile volume and area percentages to 0.
- Bile pockets contract and open/release drug to the media thus a first order bile efflux rate is available

SimulationsPlus
SCIENCE + SOFTWARE = SUCCESS

Case Study 1: Sodium Taurocholate Uptake Into Bile

SimulationsPlus
SCIENCE + SOFTWARE = SUCCESS

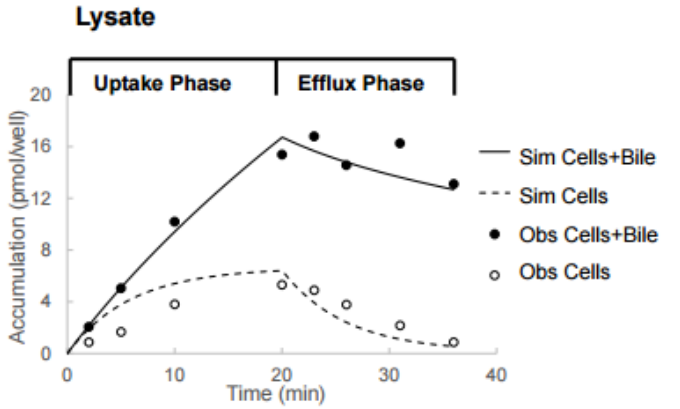
Sodium Taurocholate



AP 7.2 = ADMET Predictor v. 7.2
S+ stands for properties predicted with Simulations Plus models
S+Sw = solubility in pure water

- Experimental data from Qualyst Guo, ISSX 2014
- All properties except K_m values from ADMET Predictor

Property	Value
S+IS (mg/mL) @ pH 2.78	0.0184
S+pKa (Acid)	1.1
S+SF	906
S+pH	2.78
S+logP	0.82
S+Peff (x10-4 cm/s)	0.36
DiffCoef (x10-5 cm/s)	0.53
S+fumic	0.911



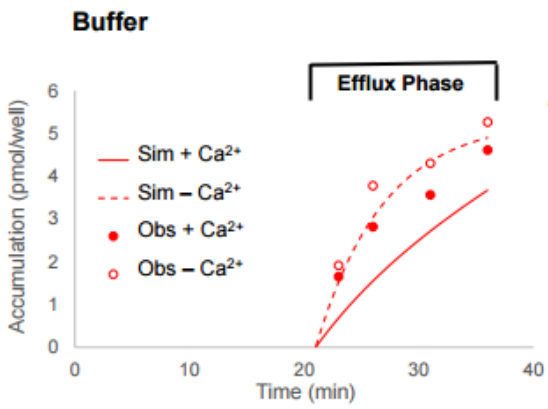
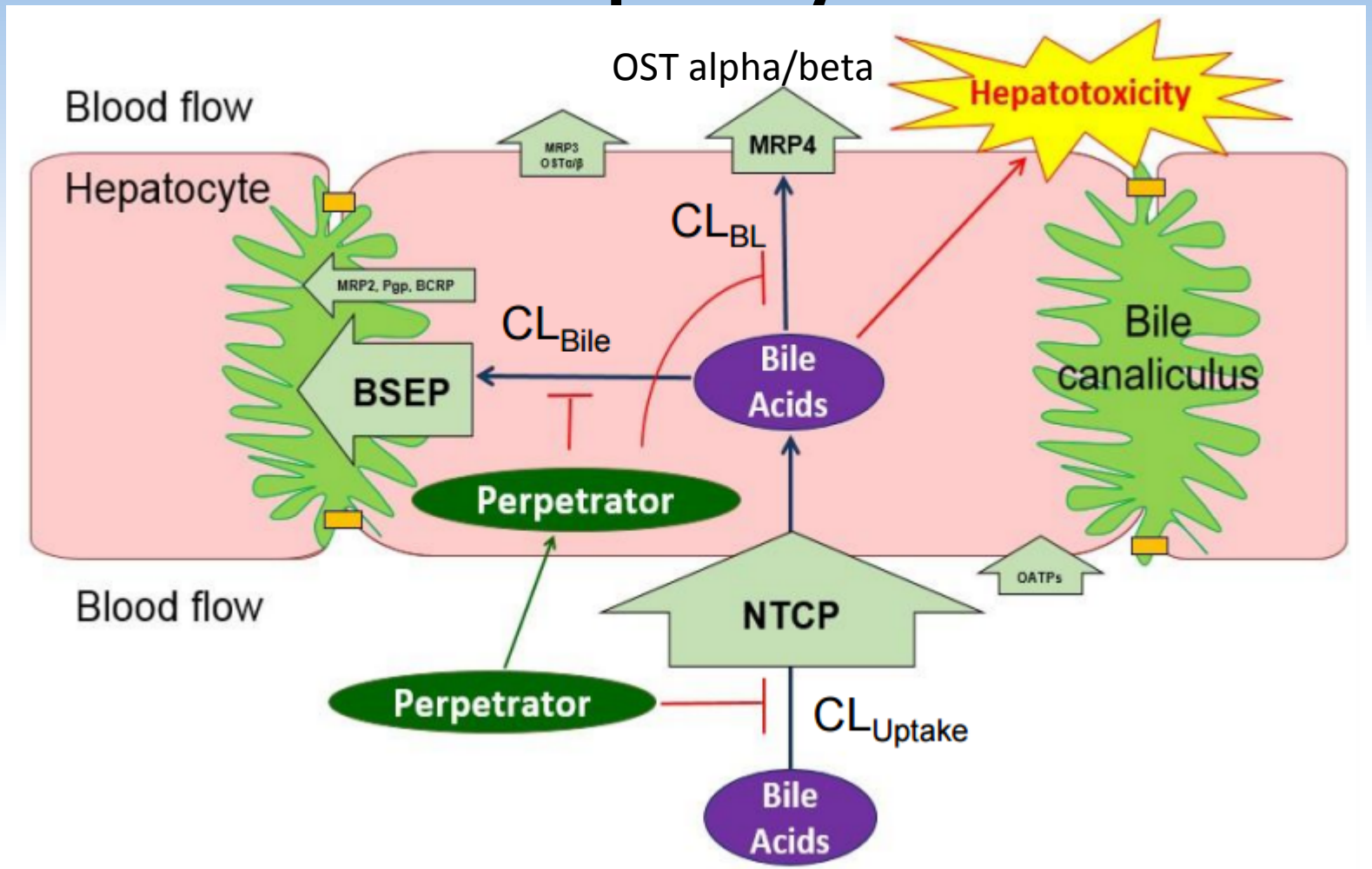


Fig. 3. Observed (circles) and simulated (lines) d_8-TCA accumulation in lysate [from cells+bile and cells] (black) and efflux buffer [Ca^{2+}-containing and Ca^{2+} - free] (red). Representative graph from one of n=3 experiments. Recovered estimates for CL (mean and SD):

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

SimulationsPlus
SCIENCE + SOFTWARE = SUCCESS

Model of Sodium Taurocholate Bile Canalicular Uptake in Sandwich Hepatocytes



- Guo, 2014 ISSX Poster

SimulationsPlus
SCIENCE + SOFTWARE = SUCCESS

Na^+ Taurocholate Model Assumptions

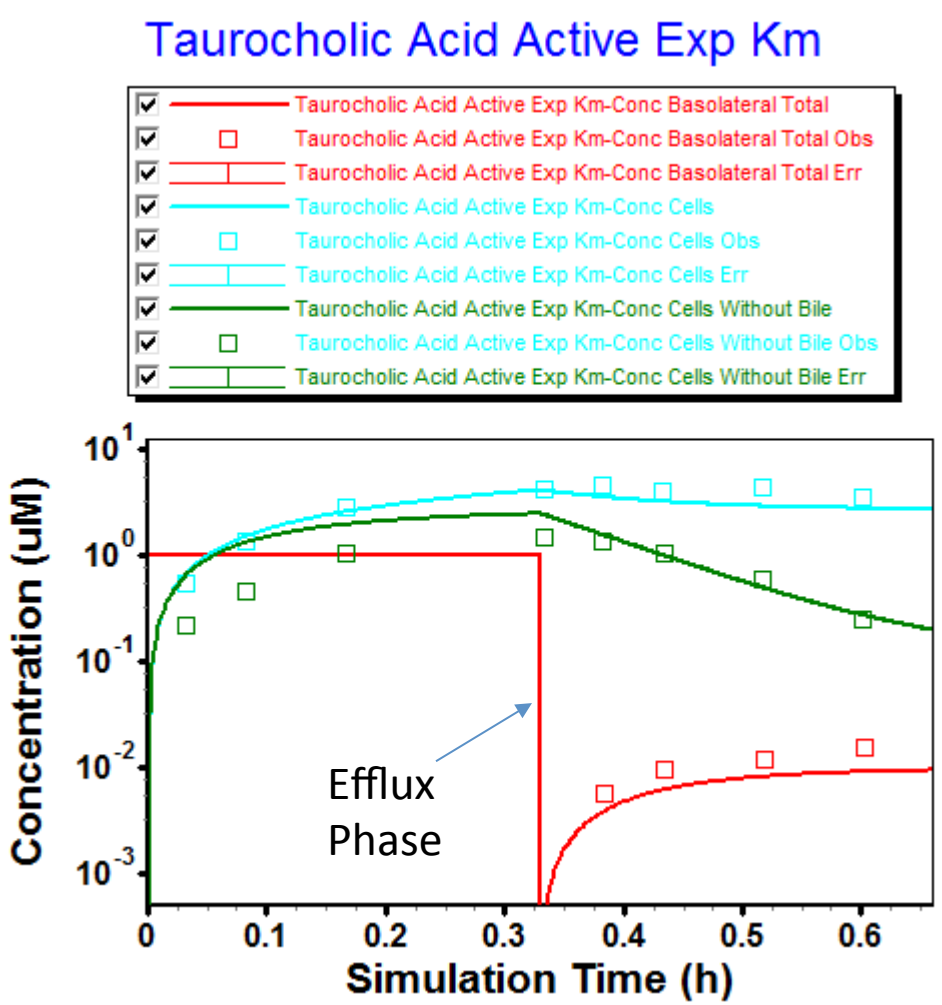
Cell Assay Inputs		
Feed Solution Conc.	1,2.5	μM
BSA	4	%
Well size	24	well
Volume	0.3	mL
Cell Vol.	6.46	pL
Cell Layer Thickness	18.6	micron
Cell Den	0.4	Mcell/well

- 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
- 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

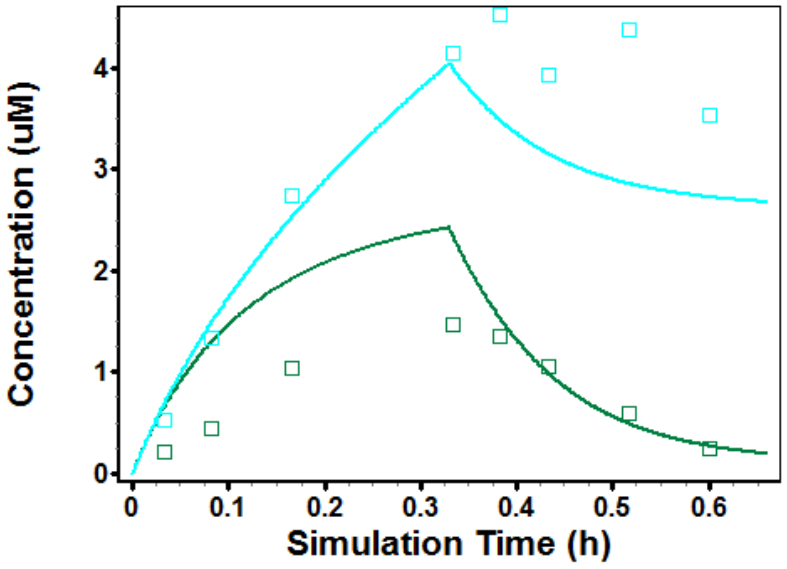
TCA Results 1 μM Donor Experimental K_m Values

- Experimental K_m values utilized from literature and V_{max} values fit to data.



- We have obtained superior model results with new datasets but they can not be shown until published.

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

SimulationsPlus
SCIENCE + SOFTWARE = SUCCESS

Quantify the Relative Importance of Influx Transport (OATP1B1) vs. Metabolism

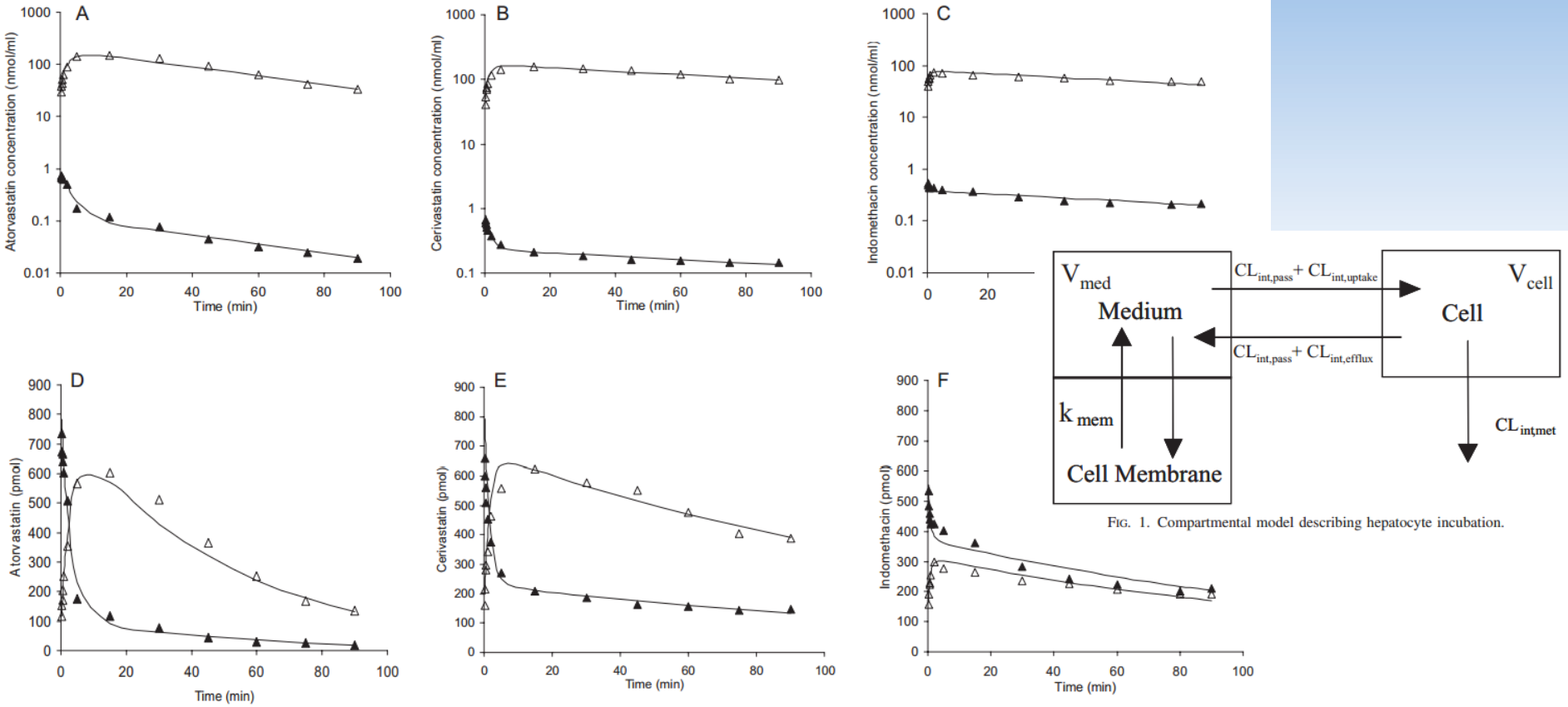


FIG. 1. Compartmental model describing hepatocyte incubation.

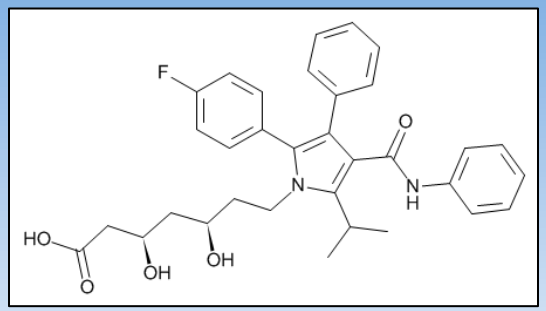
FIG. 3. Typical plots for atorvastatin (A and D), cerivastatin (B and E), and indomethacin (C and F) cell and medium data and associated WinNonlin-generated fits to the model (Fig. 1). A to C, concentration of drug in cells and medium; D to F, amount of drug in cells or medium: cells (△, cells; ▲, medium).

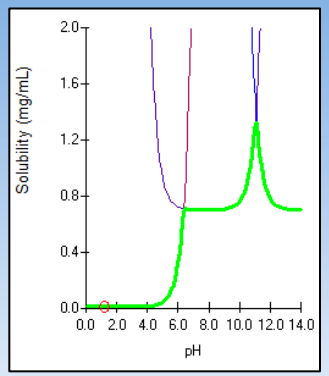
- Media and whole cell concentration data for atorvastatin, cerivastatin, and indomethacin.
- Used a simple compartmental model to extract clearance values

Paine, DMD (2008) 36:1365–1374

SimulationsPlus
SCIENCE + SOFTWARE = SUCCESS

Atorvastatin

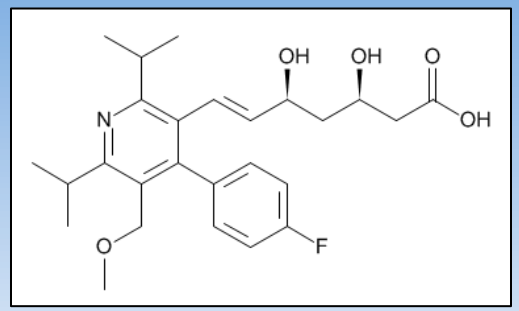


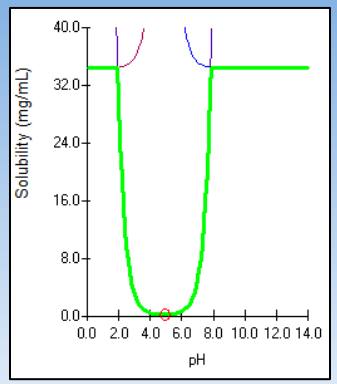


- Solubility
  - S+ Sol = 0.18 mg/mL @ pH 4.5
  - Sol (water exp) = 0.11 mg/mL
  - Sol (pH 1.2) = 0.01 mg/mL
  - Sol (pH 7.4) = 0.70 mg/mL
- S+SF = 285
  - SF (Exp) = 70
- S+ LogP 4.28
  - LogP exp = 4.18 (biobyte)
- S+ pKa (Acid) = 4.71,11.05
  - pKa Exp = 4.51 (Schonherr_Eur-J-Pharm-Biopharm_2015-155-170)
- OATP1B1 Substrate (K_m = 0.77 μM)
  Vildhede_Drug Metab_Disp_2014_42.7_1210-1218
- Caco-2 Papp (MembranePlus) = 4.80E-05 cm/s
- S+ Metabolism (2C9)
  - Experimental (3A4) (K_m = 44.9 μM)

Park_*Xenobiotica*_2008_38.9_1240-1251

Cerivastatin





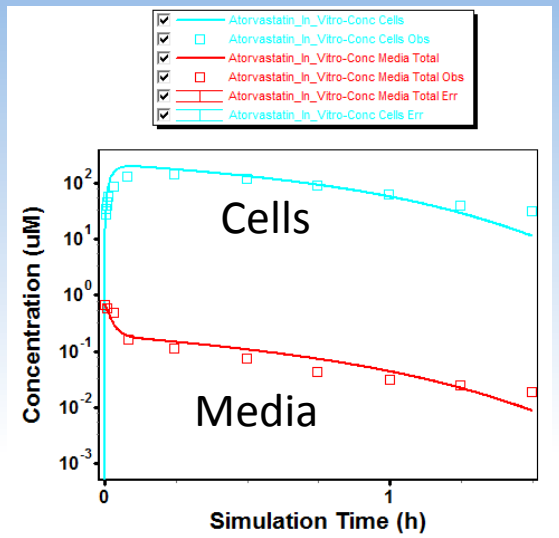
- Solubility
  - S+ Sol = 0.2 mg/mL @ pH 4.92
- S+SF = 280.7
- S+ LogP 3.04
  - LogD exp = 3.26 @ pH 5 (biobyte)
- S+ pKa (Acid/Base) = 4.39, 5.39
- OATP1B1/1A1? Substrate (K_m = 5.86 μM)
  Xenobio-Metabol-Dispos-2000-15(3)-219-225
- Caco-2 Papp (MembranePlus) = 7.5E-08 cm/s
- S+ Metabolism (3A4) (K_m = 11.8 μM)

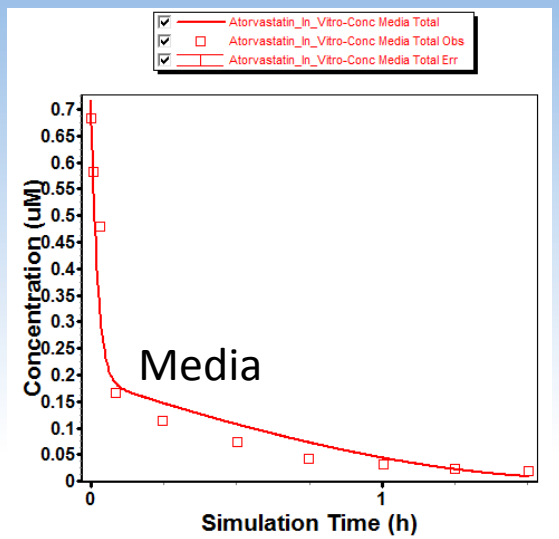
AP 7.2 = ADMET Predictor v. 7.2
S+ stands for properties predicted with Simulations Plus models
S+Sw = solubility in pure water

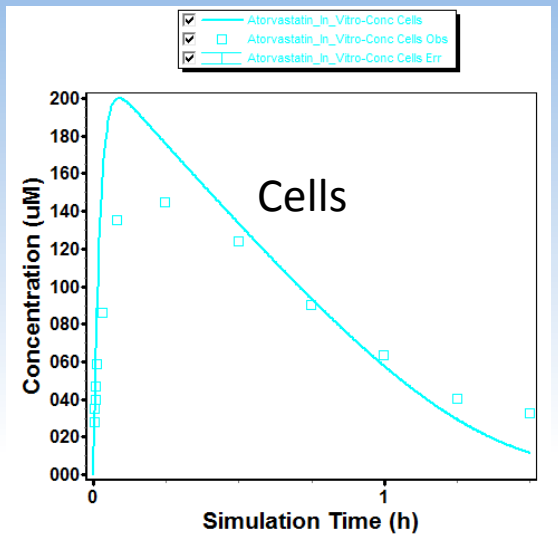
SimulationsPlus
SCIENCE + SOFTWARE = SUCCESS

Model Results

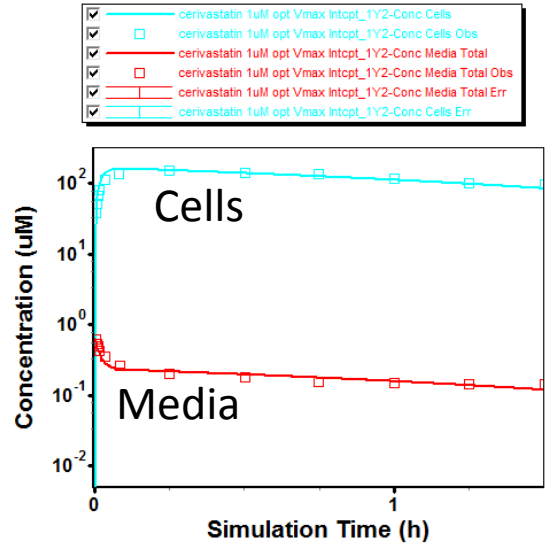
Atorvastatin

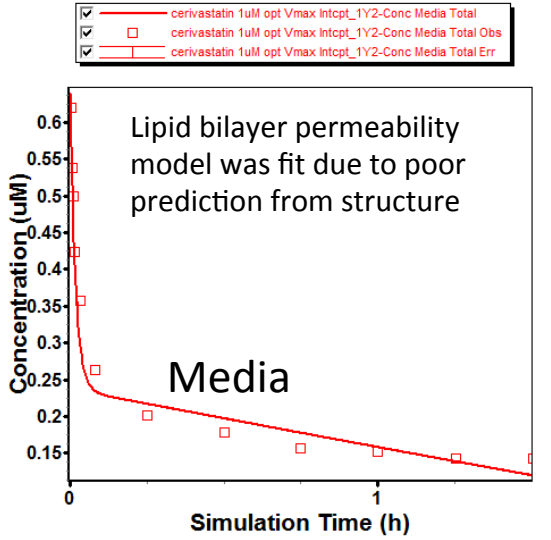


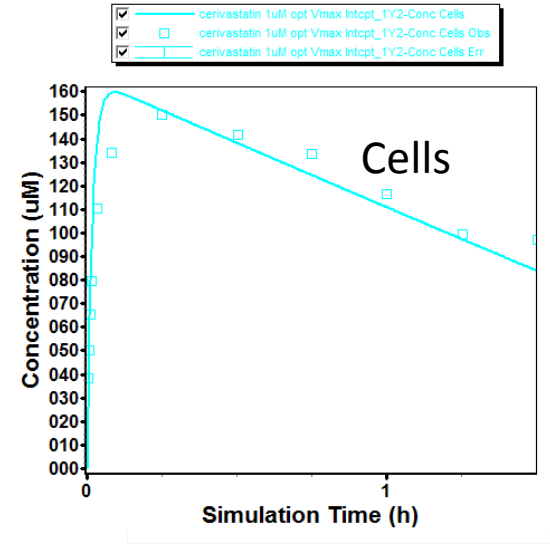




Cerivastatin







Model Results, Cont.

TABLE 2

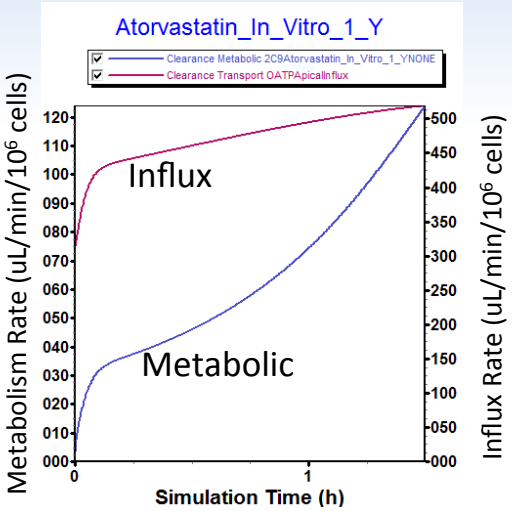
Derived and associated predicted in vitro parameters for atorvastatin, cerivastatin, and indomethacin

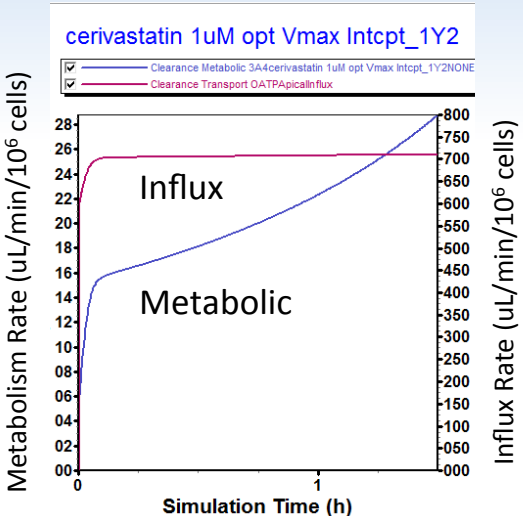
Data are mean ± S.D., and n = 3 unless otherwise stated.

	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)	[illegible]	[illegible]	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)	[illegible]	[illegible]	4.0 ± 2.3
CL_{med} (μl/min/10^6 cells)	68 ± 31	17 ± 14	7 ± 4
Vss_{med} (ml/10^6 cells)*	[illegible]	[illegible]	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.)

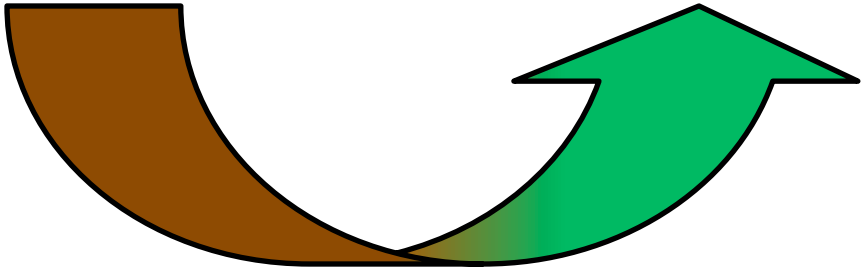
Model Comparison

- 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

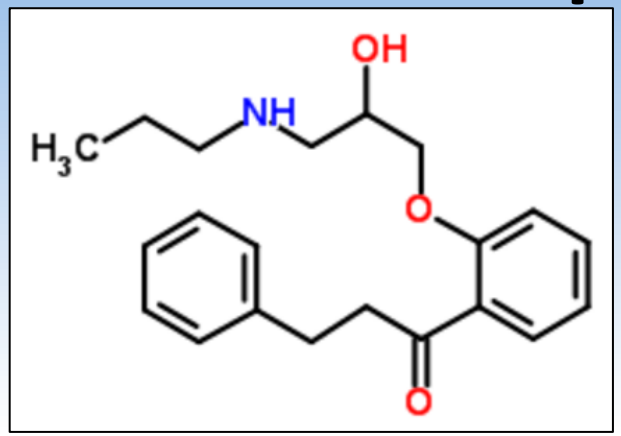
*Paine, DMD (2008) 36:1365–1374

Case Study 3: *In Vitro* to *In Vivo* Extrapolation Using MembranePlus and GastroPlus

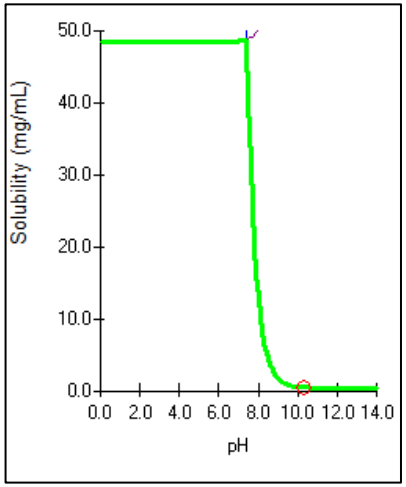


SimulationsPlus
SCIENCE + SOFTWARE = SUCCESS

Propafenone Metabolism Suspended Hepatocytes



Property	Value
S+Sw (mg/mL) @ pH 10.28	0.49
S+pKa (Base)	9.47
S+SF	114
S+logP	3.03
S+Peff (x10-4 cm/s)	1.76
DiffCoef (x10-5 cm/s)	0.65



- Reports indicate it is a CYP2D6 substrate and has saturable dose dependent kinetics.
- Have IV data to compare predicted K_m and V_{max} to.

AP 7.2 = ADMET Predictor v. 7.2
S+ stands for properties predicted with Simulations Plus models
S+Sw = solubility in pure water

SimulationsPlus
SCIENCE + SOFTWARE = SUCCESS

Propafenone Human Hepatocyte Data

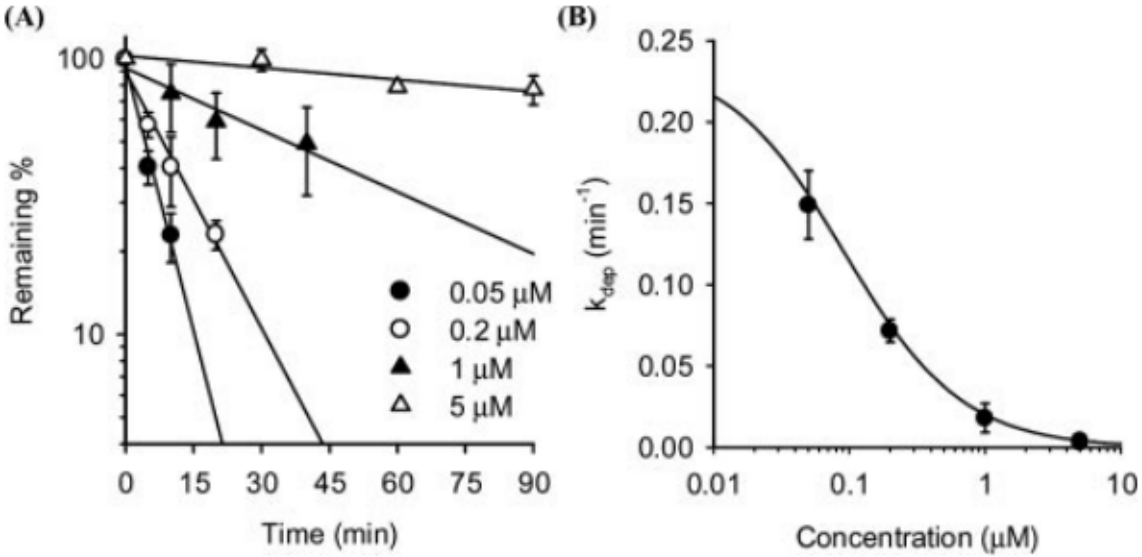


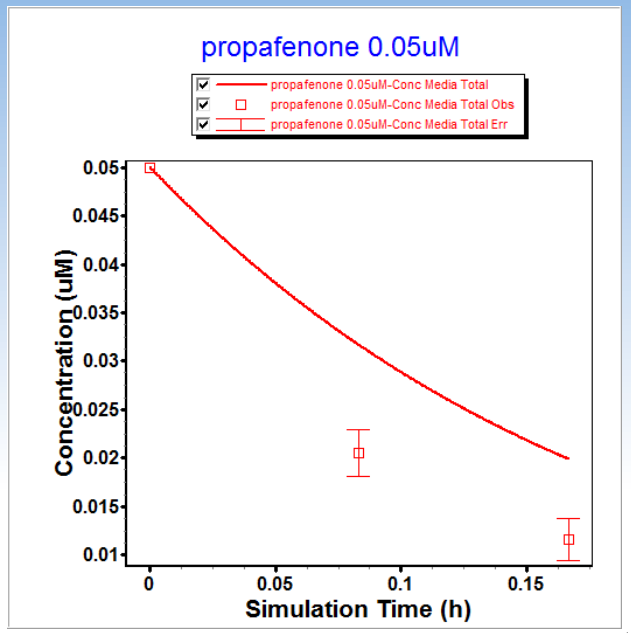
FIG. 2. A, disappearance of propafenone in cryopreserved human hepatocytes at various initial concentrations. Propafenone at concentrations of 0.05, 0.2, 1, and 5 μM was incubated with 1×10^6 cells/ml human hepatocytes individually prepared from three donors. Each point represents the mean $\pm$ S.D.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

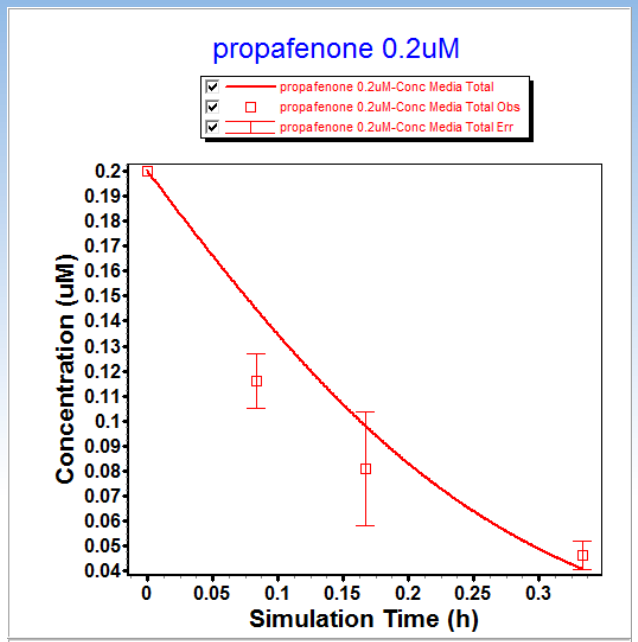
- 100 rpm
- 2 mL incubation
- 1 Mcell/mL

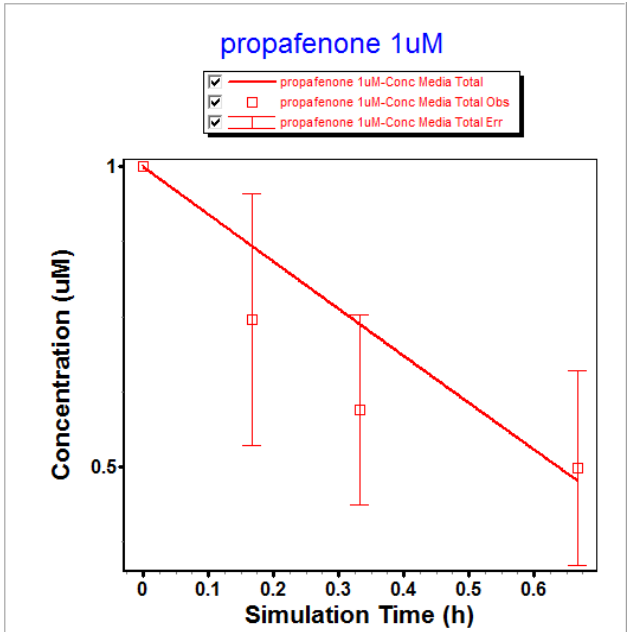
Komura, *Drug metabolism and disposition* 33.6 (2005): 726-732.

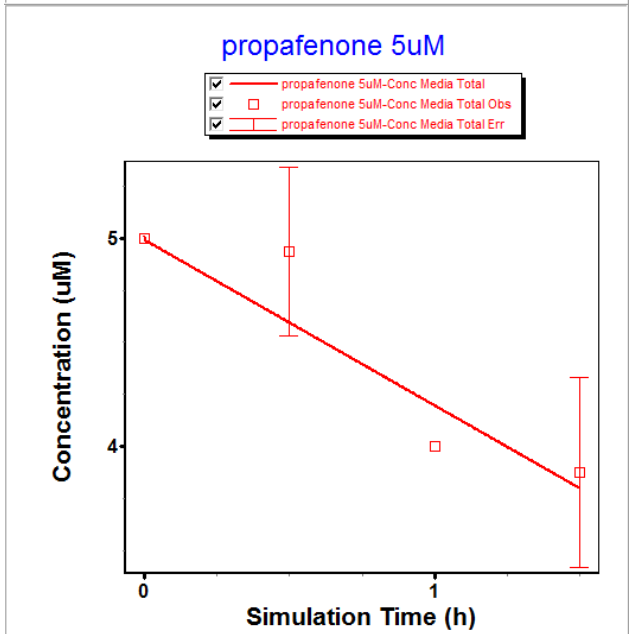
SimulationsPlus
SCIENCE + SOFTWARE = SUCCESS

Fit to *In Vitro* Data









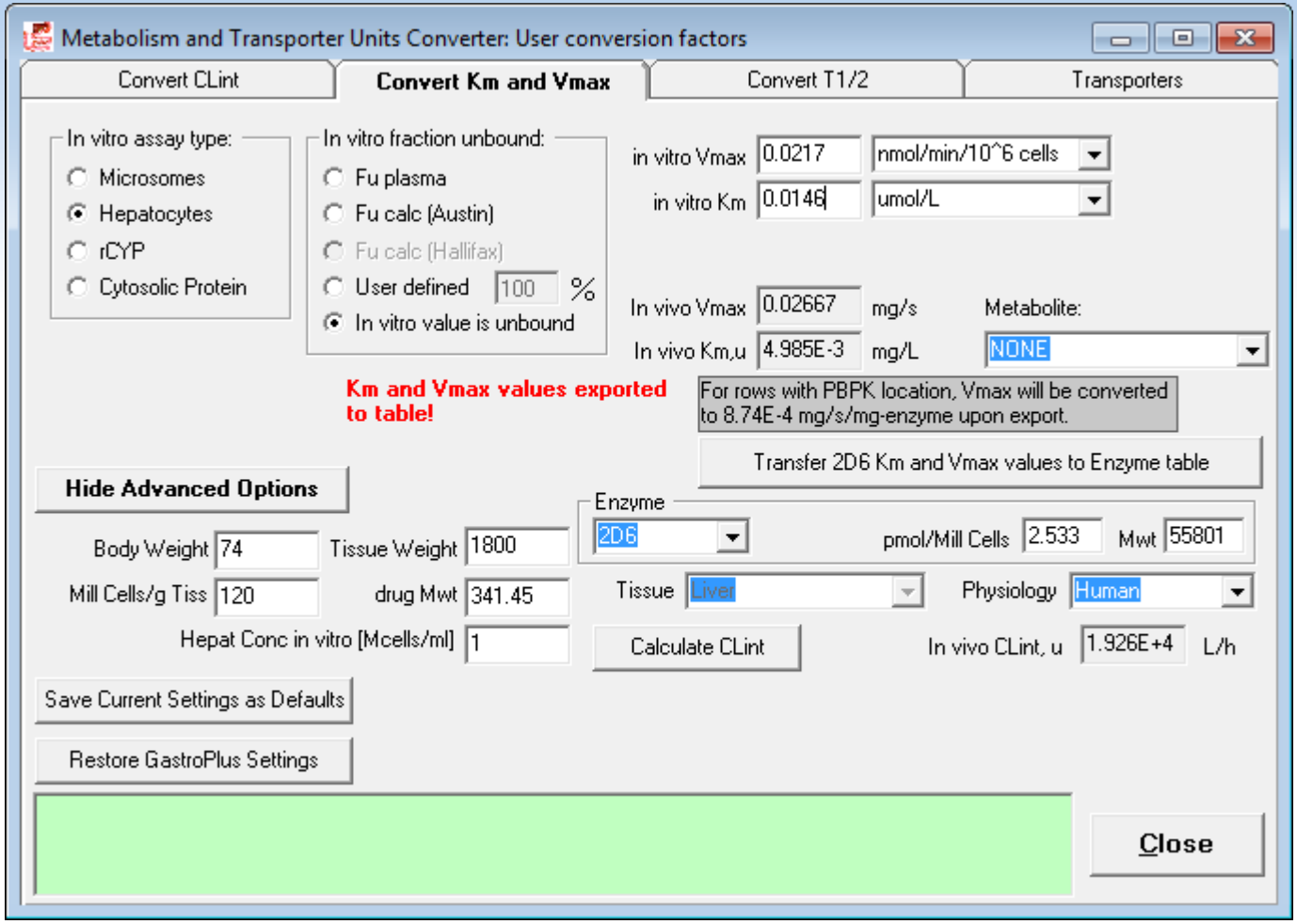
- K_m = 0.0146 µM
- V_{max} = 9.27E-02 µmol/s/L cytosol

- V_{max} Converts to :
- 2.17E-02 nmol/min/ Mcell

K_m calculated was lower than the paper which is indicative of the true intracellular K_m based on unbound intracellular concentration

SimulationsPlus
SCIENCE + SOFTWARE = SUCCESS

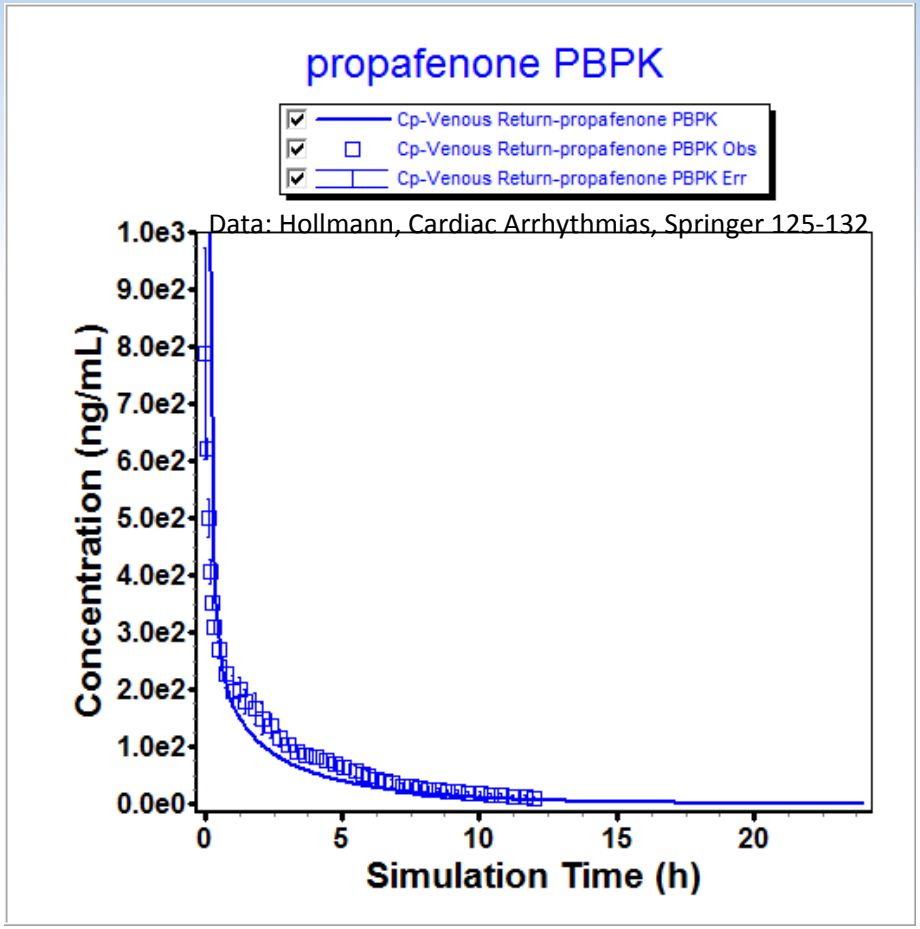
In vivo K_m / V_{max} Conversion GastroPlus

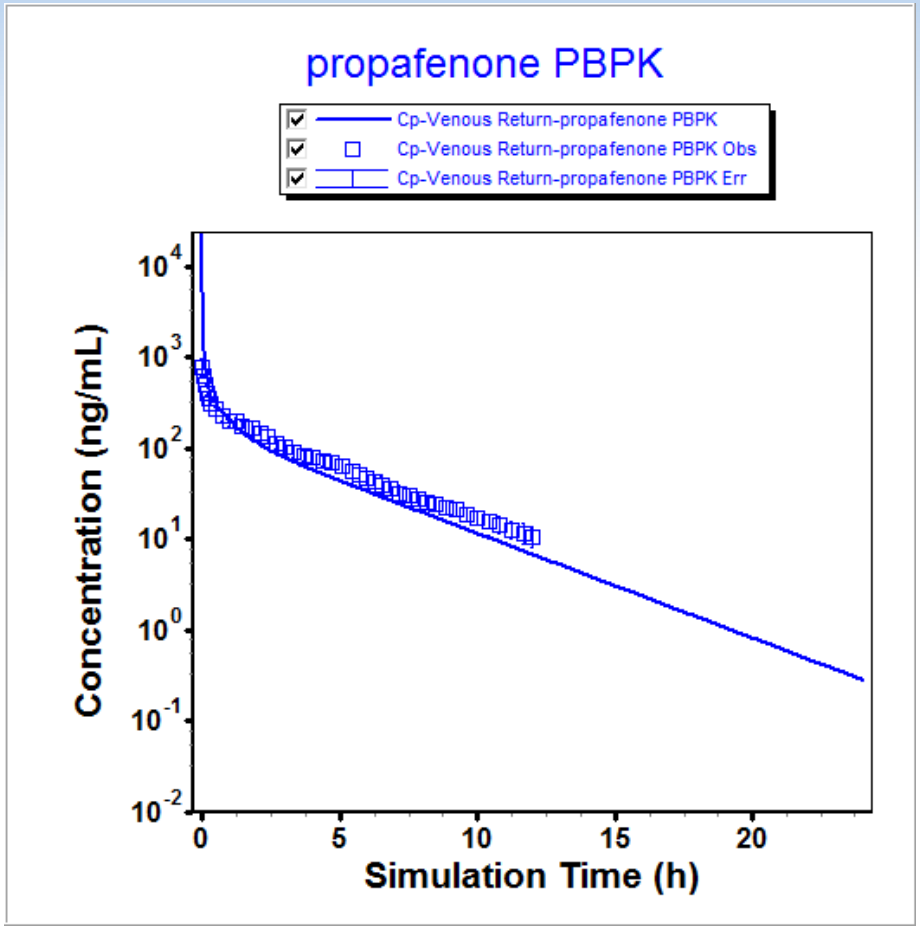


SimulationsPlus
SCIENCE + SOFTWARE = SUCCESS

Prediction of 70 mg IV Bolus Dose

- PBPK model with default parameters (Lukacova method) in GastroPlus with the *in vitro* extrapolated CYP2D6 clearance





SimulationsPlus
SCIENCE + SOFTWARE = SUCCESS

Conclusion

- MembranePlus has multiple capabilities to assess
  - Passive and active drug transport in various assays
  - Interplay between metabolism and passive/active drug transport:
    - Sandwich Hepatocytes
    - Plated Hepatocytes
    - Suspended Hepatocytes
  - Drug disposition in Bile
    - Sandwich Hepatocytes
  - Drug concentration within cellular structures
    - Lysosomes, lipid bilayers
  - Extract relevant parameters for IVIVE in GastroPlus
  - Additional validation with datasets and software release imminent