SimulationsPlus

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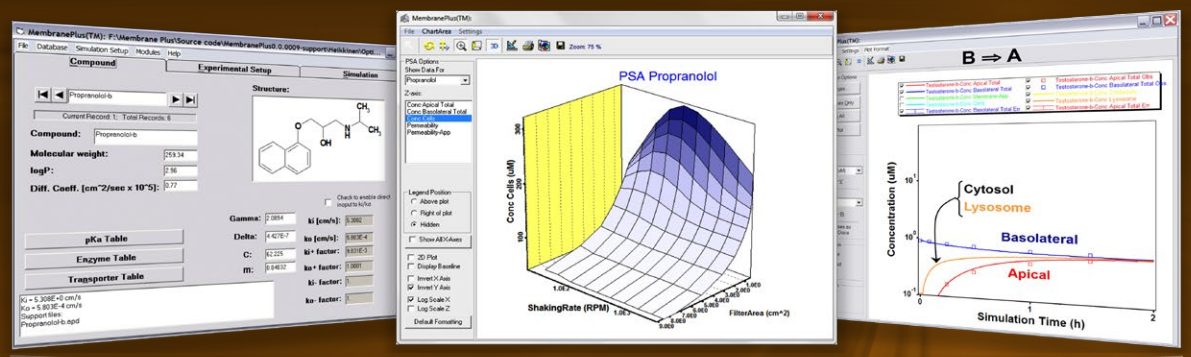
MembranePlus™ 2

Stimulate your ***kinetic*** understanding…

Permeability | Binding | Metabolism | Transporters



www.simulations-plus.com

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What is MembranePlus™?

MembranePlus is an advanced, yet easy-to-use, modeling and simulation software program that unlocks important information from your *in vitro* permeability and hepatocyte assay studies. With MembranePlus, all relevant experimental and cellular processes, such as protein binding, lysosomal trapping, pH difference, shaking rate, paracellular permeability, cell viability, and carrier-mediated transport & metabolism, are integrated to simulate drug concentrations from *in vitro* cell-based/non-cell-based assays and calculate the corresponding permeability & additional *in vivo* rate parameters. The combination of MembranePlus and GastroPlus™ brings you closer to accurately predicting *in vitro – in vivo* extrapolation (IVIVE) of absorption (both passive and carrier-mediated) and systemic clearance/distribution processes!

How can we use it?

For over 20 years, significant research has been devoted to *in vitro* permeability (e.g., Caco-2 assays) and hepatocyte systems, with several guidelines being published and all pharmacopeia describing appropriate methods for testing.

Unfortunately, these experiments are expensive, and since you don't have a lot of time or material available, how do you prioritize which compounds to test early in your project? Once you have identified a few promising leads, is your experimental design robust enough to capture all of the relevant information you need? And, after you collect the data and compile your apparent permeability or intrinsic clearance, are you certain you are not overlooking anything?

Enter MembranePlus™, the industry's leading mechanistic *in vitro* permeability & hepatocyte modeling software.

MembranePlus has several primary applications:

Simulation of *in vitro* permeability & hepatocyte concentrations

- Screen compound libraries and prioritize compounds for testing
- Predict various permeability (e.g., paracellular, transcellular) & hepatocyte (e.g., diffusional clearance, lysosomal trapping, biliary excretion) processes
- Estimate different intracellular concentrations, including:
    - o Membrane
    - o Cytosol
    - o Lysosome
- Assess impact of experimental variability on the predicted outcomes

Analysis of measured *in vitro* experimental data

- Identify paracellular vs. transcellular permeability values
- Calculate *in vitro* Km and Vmax for enzymes and transporters
- Determine the impact of lysosomal trapping
- Assess biliary excretion routes with sandwich hepatocyte assays
- Fit parameters to build a robust model and unlock important insight from the data

Who should be using it?

MembranePlus is going to be very helpful for:

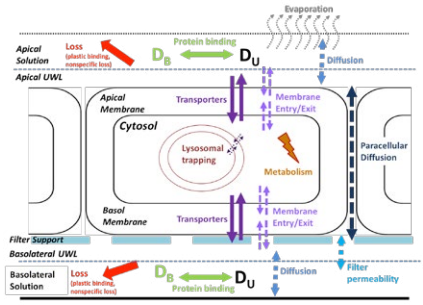
- Users of GastroPlus™ looking to better inform inputs (e.g., lysosomal trapping, diffusional clearances, biliary excretion) for more accurate *in vivo* absorption and systemic clearance/distribution predictions with the Advanced Compartmental Absorption and Transit (ACAT™) and PBPK models
- Discovery scientists looking to categorize compounds into high and low permeability classes
- DMPK researchers that focus on *in vitro* transporter/metabolism kinetic studies – model your measured data to capture all relevant information from the expensive experiments you are running
- CROs which provide *in vitro* permeability (Caco-2, PAMPA, or MDCK) or hepatocyte services, to assist with the optimization of the experimental design for client studies

Which experiments can we simulate?

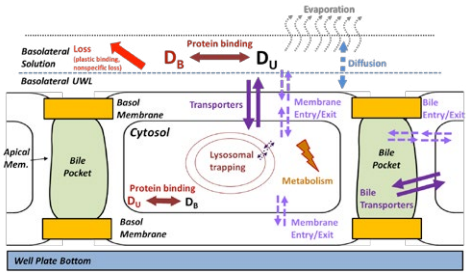
MembranePlus has default models describing several common *in vitro* systems:

1.) PAMPA (12 and 96 wells)
2.) Caco-2 (12, 24, and 96 wells)
3.) MDCK (12, 24, and 96 wells)
4.) **NEW!** Sandwich hepatocytes (6,12, 24, 48, and 96 wells)
5.) **NEW!** Suspended hepatocytes (6, 12, 24, 48, and 96 wells)

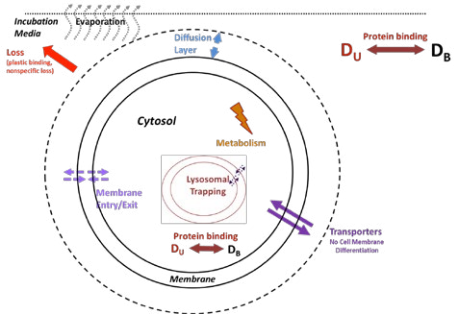
Permeability assays



Sandwich hepatocytes



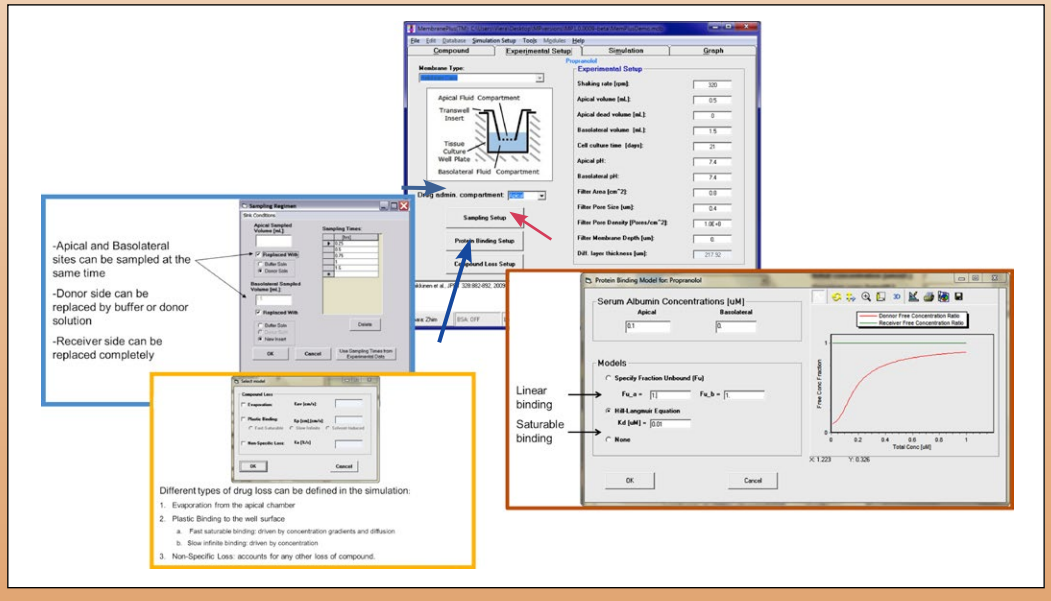
Suspended hepatocytes



Of course, use MembranePlus to customize any cell-monolayer based *in vitro* system to mimic your in-house lab!

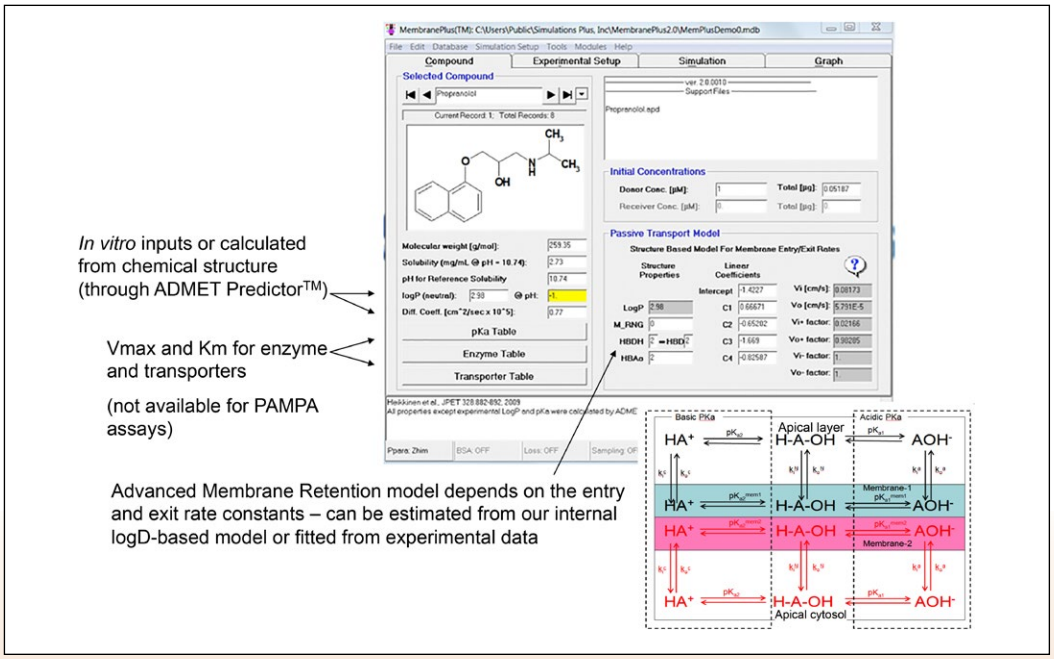
For the experiment, MembranePlus allows you to control the following during your simulations:

1.) pH in donor and receiver compartments
2.) Addition of albumin to donor or receiver compartments
3.) Shaking/stirring rates
4.) Volume of media in donor and receiver compartments
5.) Sampling protocols that may impact volume or drug concentrations, including:
    a. Withdrawal of sample volume
    b. Replacement of sample volume with blank buffer or original donor solution
    c. Moving the insert to a different plate with blank buffer in receiver compartment
6.) Cell culture time
7.) Filter surface area
8.) Filter pore size and density
9.) Cell viability and density



What drug information is needed for the simulation?

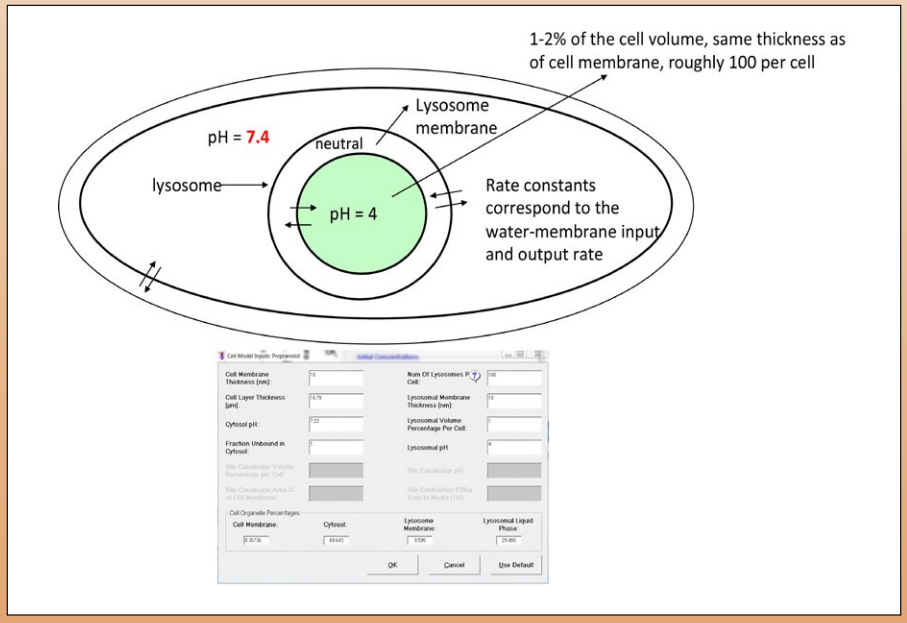
Basic physicochemical properties (e.g., logP/logD, pKa(s), and molecular weight) can be defined through *in vitro* measurements or predicted from chemical structure (through ADMET Predictor™). If including enzymes or transporters in your assay, information about Vmax and Km needs to be entered (or can be "fitted" with measured concentration-time data from your permeability or hepatocyte studies).



Lysosomal Trapping - An important consideration

In MembranePlus, lysosomal trapping, a process which can impact a compound's distribution in tissues, can be studied in the simulations.

NEW! Fu Cell – this new output provides an estimate of Fu,Enterocytes for use in GastroPlus PBPK models. This improves the prediction of absorption & pharmacokinetics for compounds exhibiting lysosomal trapping and/or high binding in enterocytes causing extended Tmax values.

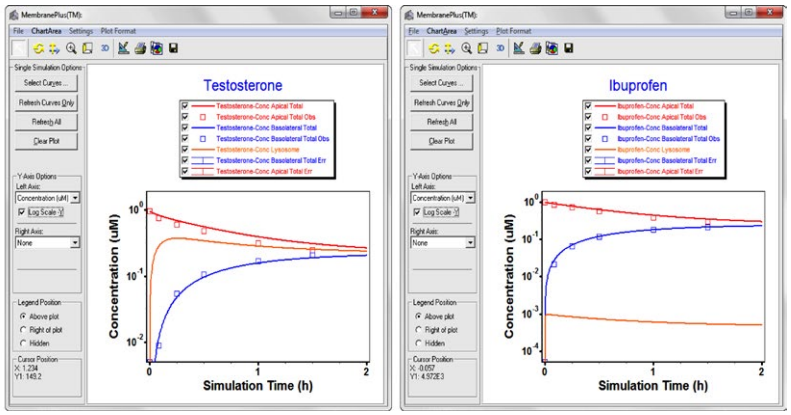


The Simulation Outputs - how does it look?

With MembranePlus, you can run the program in one of several modes:

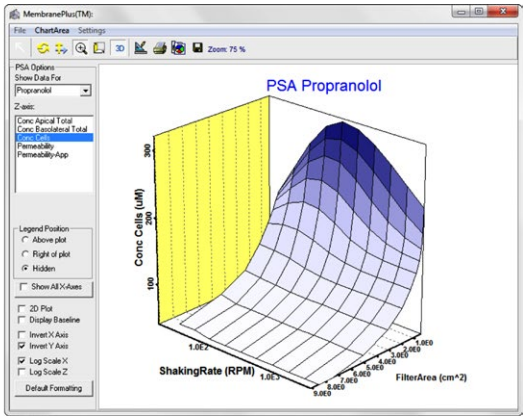
- **Single simulation:** based on your drug properties (whether measured or predicted through ADMET Predictor™) and experimental setup, easily run a simulation to predict the time course changes in concentrations (e.g., apical, basolateral, cellular, or lysosomal).

After running single simulations, view changes in concentrations (e.g., total apical, basolateral, cellular or lysosomal concentrations) vs. time



- **Parameter Sensitivity Analysis:** during early drug development, researchers have a large number of compounds to evaluate and limited resources. With Parameter Sensitivity Analysis, quickly assess the impact of changes to certain properties (e.g., physicochemical or experimental) on critical endpoints. This can help guide your resource allocation plans and identify which experiments should be done next.

With Parameter Sensitivity Analysis, view changes in endpoints (e.g., total apical, basolateral or cellular concentrations) vs. selected parameters (in this figure, shaking rate and filter surface area)



- **Batch Simulations:** quickly screen a library of compounds based on predicted permeability or run the same compound through a series of different Caco-2, PAMPA, MDCK, or hepatocyte experiments. Easily prioritize compounds in any way you like: from high to low permeability, based upon potential issues with lysosomal trapping, etc.

- **Optimization:** an important feature of MembranePlus which turns the program into a fitting routine. Using your measured concentration vs. time data, fit parameters to build more robust models and "learn" from your chemical series. Or, apply the feature to help design your *in vitro* permeability (Caco-2, PAMPA, MDCK) or hepatocyte experiment

Regardless of the mode in which you run MembranePlus, report-quality results can be easily generated and shared with others.

T2002

MembranePlus™: A Tool to Study *In Vitro*/*In Vivo* Transport and Drug-drug Interaction

Ke X. Szeto, Viera Lukacova, Walter S. Woltosz, and Michael B. Bolger

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PURPOSE

To develop a mechanistic mathematical model for analysis of *in vitro* permeability assays that accounts for all mechanisms contributing to observed apparent permeability: passive paracellular and transcellular diffusion, carrier-mediated transport, as well as drug accumulation in membranes and some intracellular compartments (e.g., lysosomes). The model was validated by analyzing the effect of competitive inhibition of P-gp by vinblastine on the apparent permeability of indinavir in Caco-2 monolayers.

METHODS

MembranePlus™ (Simulations Plus, Inc.) was used to analyze the concentration-time profiles in donor and receiver compartments after apical and basolateral administration of 50 µg/mL (81.5 µM) indinavir alone and co-administration of 50 µg/mL (81.5 µM) indinavir with 70 µM vinblastine [1]. The physicochemical properties of indinavir and vinblastine were predicted by ADMET Predictor™ 6.0 (Simulations Plus, Inc.). The contribution of paracellular diffusion was estimated from drug properties and the experimental setup. Carrier-mediated transport was modeled with Michaelis-Menten kinetics. The indinavir P-gp Vmax/Km ratio, along with parameters accounting for passive transcellular diffusion and membrane accumulation were fitted to the indinavir-alone data. This basic model was then applied to simulate the inhibition of P-gp by vinblastine by fitting the Vmax/Km ratio for indinavir with competitive inhibition of P-gp by vinblastine. The model also includes various effects of major experiment-related parameters (e.g, shaking rate, solvent pH, filter support and sampling effects).

1. Compound Property

Figure 2. MembranePlus compound tab. The program allows two ways of accounting for passive diffusion through cell membranes: (1) S+ model as listed in Eqn. 1 and 2; and (2) direct transport parameter entries for all neutral and charged species.

The membrane entrance and exit rates are given by Eqn. 1 and 2, as modified from [2] and [3]

$$k_i = \frac{\gamma P_{mem}}{\delta P_{mem} + 1}, k_o = \frac{\gamma}{\delta P_{mem} + 1} \quad \text{Eqn. 1}$$

$$P_{mem} = c(P_{oct})^m \quad \text{Eqn. 2}$$

2. Transporter Setup

A P-gp efflux transporter is set up in the program transporter table.

3. Experimental Setup

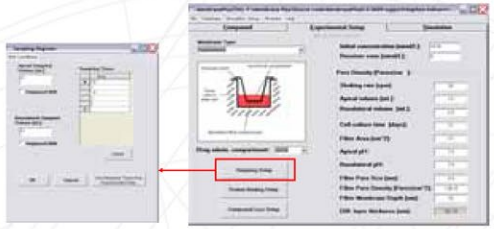

Figure 3. MembranePlus Experimental Setup tab. The program considers major experiment-related parameters, such as shaking rate, apical volume, basolateral volume, filter area, and filter support permeability and etc. As in [1], 0.1 mL samples were drawn from both and basolateral chambers at 0.5, 1, 1.5, 3 and 5 hours.

4. Paracellular and filter permeability

Both paracellular and filter permeabilities are accounted for in the program. The default paracellular model is the Zhimin model [4], which accounts for the molecule mean projected radius and the hydrodynamically equivalent sphere radius. The filter permeability calculation was adopted from [5]. Therefore, the effective paracellular permeability is given by

$$\frac{1}{P_{para}^{eff}} = \frac{1}{P_{para}} + \frac{1}{P_{Filter}} \Rightarrow P_{para}^{eff} = \frac{P_{para} P_{Filter}}{P_{para} + P_{Filter}}$$

where

$$P_{Filter} = \frac{\varepsilon_F * D * F(r/R_F)}{h_F},$$

$F(x) \rightarrow$ Renkin Function

RESULTS

R^2 for indinavir-alone data was 99.2% (A->B) and 88% (B->A) and R^2 for vinblastine inhibition data was 91.1% (A->B) and 85.1% (B->A). The ratio between the two transport fluxes has been calculated to be ~50, indicating a strong P-gp inhibition effect by vinblastine.

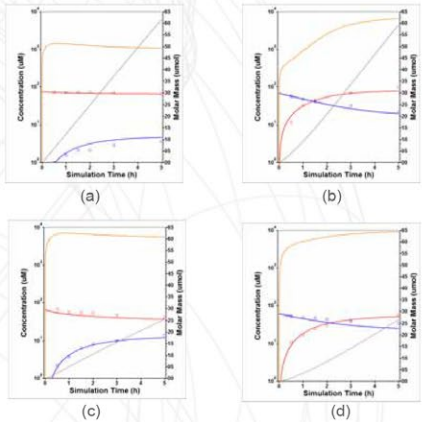

Figure 3. 5-hour simulation of indinavir profiles after (a) apical administration of indinavir, (b) basolateral administration of indinavir, (c) apical administration of indinavir and vinblastine and (d) basolateral administration of indinavir and vinblastine. Red: apical concentration; blue: basolateral concentration; orange: lysosomal concentration; purple: amount transported by P-gp transporter.

CONCLUSIONS

MembranePlus accurately simulated the results of *in vitro* experiments with respect to a variety of mechanisms affecting measured apparent permeability. It is a promising tool in drug research and development. By separating the system-specific from drug-specific parameters in description of drug permeation through the cell membranes it allows obtaining "clean" drug-specific properties (i.e. intracellular Km for efflux transporters) that will allow more direct *in vitro-in vivo* extrapolation and predictions of absorption and drug-drug interactions.

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References:

[1] S. Kapitza et al, European Journal of Pharmaceutics and Biopharmaceutics 66, 2007, 146-158
[2] S. Balaz et al., General Physiology and Biophysics 6, 1987, 65-77
[3] H. Kubinyi et al., Journal of Pharmaceutical Sciences 67 (2), 1977, 262-3
[4] H. Zhimin et al., Transactions of Tianjin University 1 (1), 1995, 42-47
[5] A. Adson et al., Journal of Pharmaceutical Sciences 84 (10), 1995, 1197-1204

MembranePlus™: A tool to Study *in vitro/in vivo* Transport and Lysosomal Trapping

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PURPOSE

To develop a mechanistic mathematical model for analysis of *in vitro* permeability assays that accounts for all mechanisms contributing to observed apparent permeability: passive paracellular and transcellular diffusion, ionization effects, carrier-mediated transport, and metabolism, as well as drug accumulation in membranes and some intracellular compartments (e.g., lysosomes). The model was applied to analyze the effects of lysosomal trapping on the measured apparent permeability of propranolol.

METHODS

MembranePlus™ (Simulations Plus, Inc.) was used to analyze the concentration-time profiles in donor and receiver compartments after apical and basolateral administration of ibuprofen, testosterone and propranolol alone, as well as propranolol in the presence of bafilomycin [1]. The physicochemical properties of propranolol, ibuprofen, and testosterone were predicted by ADMET Predictor™ 6.0 (Simulations Plus, Inc.). The contribution of paracellular diffusion for each drug was estimated from drug properties and the experimental setup. Parameters accounting for passive transcellular diffusion and membrane/lysosomal accumulation were fitted to the propranolol, ibuprofen, and testosterone alone data. This basic model was then applied to explore the effect of bafilomycin on lysosomal pH and the subsequent change in propranolol accumulation in lysosomes. The model includes various effects of the experimental setup (i.e., shaking rate, solvent pH, filter support, sampling effects, etc.) on measured apparent permeability.

1. Compound Properties

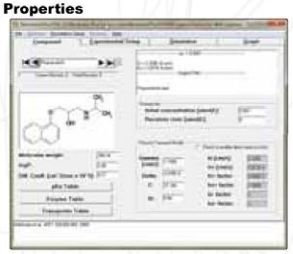


Figure 1. MembranePlus compound tab. The program allows two ways of accounting for passive diffusion through cell membranes: (1) the S+ model as shown in Eqn. 1 and 2; and (2) direct transport parameter entries for all neutral and charged species.

The membrane entrance (Ki) and exit rates (Ko) are given by Eqn. 1 and 2, as modified from [2] and [3], where P_{oct}, P_{mem} are the octanol-water and membrane-water partition coefficients, and c, m, γ and δ are fitting coefficients.

$$k_i = \frac{\gamma P_{mem}}{\delta P_{mem} + 1}, k_o = \frac{\gamma}{\delta P_{mem} + 1} \qquad \text{Eqn. 1}$$

$$P_{mem} = c(P_{oct})^m \qquad \text{Eqn. 2}$$

2. Experimental Setup

Figure 2. MembranePlus Experimental Setup tab. The program considers major experiment-related parameters, such as shaking rate, apical volume, basolateral volume, filter area, filter support permeability, etc.

3. Diffusion Model

The unstirred layer thicknesses are automatically calculated after the relevant experimental parameters are entered: shaking rate, apical and basolateral volumes, filter area. Therefore, in the current program, the apical and basolateral departments are separated into a well-stirred layer and a diffusion layer. This diffusion layer is then divided into a number of thinner sublayers to allow accurate calculation of diffusion across the layers according to Fick's Second Law, where c is the concentration and D is the diffusion coefficient.

$$\frac{\partial c}{\partial t} = D\frac{\partial^2 c}{\partial x^2} \qquad \text{(Fick's Second Law)}$$

4. Paracellular and filter permeability

Both paracellular and filter permeabilities are accounted for in the program. The default paracellular model is the Zhimin model [4], which accounts for the molecule's mean projected radius and the hydrodynamically equivalent sphere radius. The filter permeability calculation was adopted from [5]. The total effective paracellular permeability is given by

$$\frac{1}{P_{para}^{eff}} = \frac{1}{P_{para}} + \frac{1}{P_{Filter}} \Rightarrow P_{para}^{eff} = \frac{P_{para}P_{Filter}}{P_{para} + P_{Filter}}$$

where

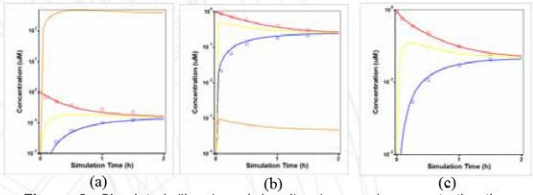
$$P_{Filter} = \frac{\varepsilon_F * D * F(r/R_F)}{h_F},$$

$$F(x) \rightarrow \text{Renkin Function}$$

where P_{para} and P_{Filter} are the paracellular and filter permeabilities, r is the molecular radius, R_F is the filter pore radius, ε_F is the filter porosity and h_F is the filter pore depth.

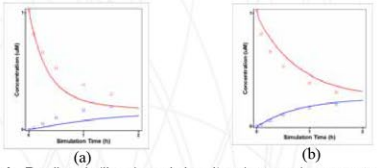
Results

The membrane transport model (c, m, γ and δ) was fitted to apical and basolateral administrations of ibuprofen, testosterone and propranolol simultaneously (6 profiles). The fitted concentration-time profiles showed a good match to the experimental data (Figure 3). The overall R^2 for all the concentrations was over 0.9. The same model was then applied to co-administration of propranolol and bafilomycin, suggesting that bafiloimycin caused an increase in lysosomal pH from 4 to 5.5 (Figure 4), which resulted in a good match of propranolol concentration-time profiles measured in the presence of bafilomycin.



(a) (b) (c)

Figure 3. Simulated (lines) and *in vitro* (squares) concentration-time profiles after apical administration of 1 μM (a) propranolol (b) ibuprofen and (c) testosterone. Simulated apical, basolateral, cytosol and lysosomal concentrations are shown in red, blue, yellow and orange respectively. Note that in (c), cytosol and lysosomal concentrations are overlapping. A similar match between simulated and observed profiles was obtained for basolateral administration (data not shown).



(a) (b)

Figure 4. Predicted (lines) and *in vitro* (squares) concentration-time profiles after apical administration of 1 μM propranolol in the presence of 100 nm bifilomycin. (a) lysosomal pH set to be 4. (b) lysosomal pH set to be 5.5. Simulated apical, basolateral concentrations are shown in red and blue, respectively. A similar match between simulated and observed profiles was obtained for basolateral administration (data not shown).

CONCLUSIONS

MembranePlus has enabled the ability to analyze *in vitro* experiments with respect to the variety of mechanisms affecting measured apparent permeability. The current study shows that it is a promising tool in drug research and development for *in vitro-in vivo* extrapolation, not only in prediction of absorption, but also other processes affecting drug distribution in different tissues (e.g., lysosomal trapping).

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References:

[1]A.T. Heikkinen, JPET 328, 2009, 882-892
[2] S. Balaz et al., General Physiology and Biophysics 6, 1987, 65-77
[3] H. Kubinyi et al., Journal of Pharmaceutical Sciences 67 (2), 1977, 262-3
[4] H. Zhimin et al., Transactions of Tianjin University 1 (1), 1995, 42-47
[5] A. Adson et al., Journal of Pharmaceutical Sciences 84 (10), 1995, 1197-1204

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