Key capabilities of the SIVA Version 3 Permeability/Transport module

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The user friendly **S**imcyp **I**n-**V**itro **A**nalysis (**SIVA**) Toolkit Version 3 permeability/transport module has some unique capabilities:

Transporter kinetic analysis (K_m, J_{max})

Data for bidirectional transport of vinblastine and verapamil across monolayers with different level of expression of P-gp were previously generated [1-2]. The data were first analysed with a conventional approach using the nominal donor concentration as the driving force. The same data were reanalysed using a three-compartment (3C) model developed in SIVA, in which case the driving concentration for efflux was intracellular and sink conditions were not assumed.

The K_m estimates from the conventional approach changed with the level of P-gp protein expression, whereas K_m estimates obtained using the 3C model were lower and independent of P-gp level (Table 1). The parameter estimates from SIVA were similar to those published in literature using a similar modelling approach (Table 1).

Table 1. SIVA estimated K_m values compared to conventional analysis and literature:

	Conventional Analysis[5-7] K_m (µM)	3C model K_m (µM, 95% CI) SIVA	3C model K_m (µM) Literature [2]
Vinblastine			
Highly P-gp induced Caco-2	251	1.64 (0.83 - 2.5)	1.07
P-gp induced Caco-2	163	2.38 (1.2 – 3.6)	3.04
'Normal' Caco-2	102	2.79 (1.6 – 3.9)	3.97
MDR knockdown Caco-2	31	1.65 (1.2 – 2.1)	1.61
Verapamil			
MDR-MDCKII cells	3.95	0.717 (0.30 – 1.2)	0.670
Highly P-gp induced Caco-2	1.93	0.489 (0 – 1.4)	0.388
P-gp induced Caco-2	1.28	0.375 (0 – 0.86)	0.334
'Normal' Caco-2	0.82	0.354 (0 – 1.2)	0.327

Electrochemical gradient driven (EGD) transport

The kinetics of metformin uptake into HEK293 cells expressing hOCT2 and plasmid vector alone (mock cells) were determined [3]. The amount of metformin in cell lysates were calculated from scintillation count data and converted to a cell concentration on the basis of protein concentration and an estimate of HEK293 cell volume (6.4 µL/mg protein [4]).

Cell concentration data were modelled using a two-compartment (media and cell) model in which OCT2 transport was defined by either conventional Michaelis-Menten kinetics or by an electrochemical gradient driven (EGD) transport model. The passive permeability of metformin was determined by fitting data from mock cells, with the resulting CL_{PD} value fixed when fitting data from OCT2-HEK293 cells.

With the EGD model (below), the rate of OCT2 mediated transport was defined based on the electrochemical gradient of metformin across the cell membrane. This model was previously used to fit metformin uptake data at a single concentration and also applied in a PBPK model [5]. The J_{OCT2} parameter was fitted, whilst the membrane potential was also fitted within bounds defined by experimental values in HEK cells.

$$rate\ of\ OCT2\ mediated\ uptake = -\Phi_{df} \cdot J_{OCT2} \cdot no.cells$$

$$\Phi_{df} = \Phi_m - \frac{R \cdot T}{z \cdot F} \cdot ln\left(\frac{[S]_{media}}{[S]_{cell}}\right)$$

Where J_{OCT2} is the rate (pmol/min/volt/million cells) of OCT2 mediated transport, Φ_{df} is the electrochemical driving force (Volts), Φ_m is the membrane potential (Volts), R is the universal gas constant (8.314 Joules.Kelvin^{-1}. mol^{-1}), T is temperature (310.15 Kelvin in the experiment at 37°C), z is the valence of the ionic species (+1 for metformin) and F is Faraday's constant (96490 coulombs.mol^{-1}).

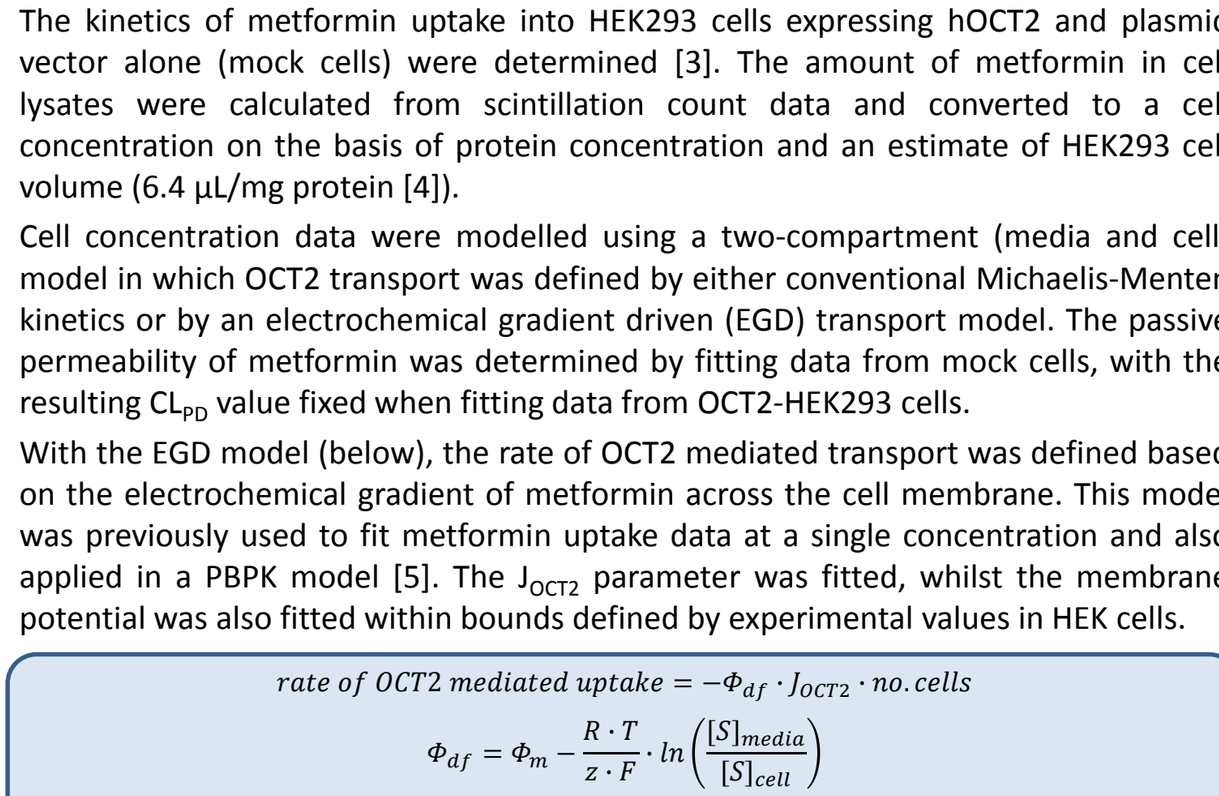

Figure 1. Observed (points), Michaelis-Menten fitted (dotted lines) and EGD-model fitted (solid lines) metformin concentrations in OCT2-HEK293 cells at different initial buffer metformin concentrations.

Sampling options for Transwell assays

Data for the bidirectional transport of metoprolol across Caco-2 monolayers was previously generated [6]. Caco-2 cells were seeded onto 12-well Transwell inserts and grown for 20 days. Transport experiments were performed at buffer (pH 7.4:7.4) volumes of 0.5 and 1.5 mL in apical and basolateral compartments, respectively. Experiments (n = 6 filters) were initiated by adding [^{3}H]-metoprolol to donor buffer at a concentration of 1.1 µM. At 5, 15, 25, 50, 80 and 120 mins, receiver buffer was sampled. Sampling of A to B experiments was conducted by moving the Transwell insert to a new well containing blank buffer and retaining the previous well, thereby representing complete removal of drug from basolateral buffer. Sampling of B to A experiments was conducted by removal of 400 µl of apical buffer and replacement with an equal volume of blank buffer. [^{3}H]-metoprolol concentrations in the sampled buffer were determined by scintillation counting.

The receiver well concentration data were fitted with the three-compartment model in SIVA with the impact of sampling dynamically accounted for within the model. The estimated (single membrane) passive permeability was 253 x 10^{-6} cm/s (95 % CI 247-259 x 10^{-6} cm/s).

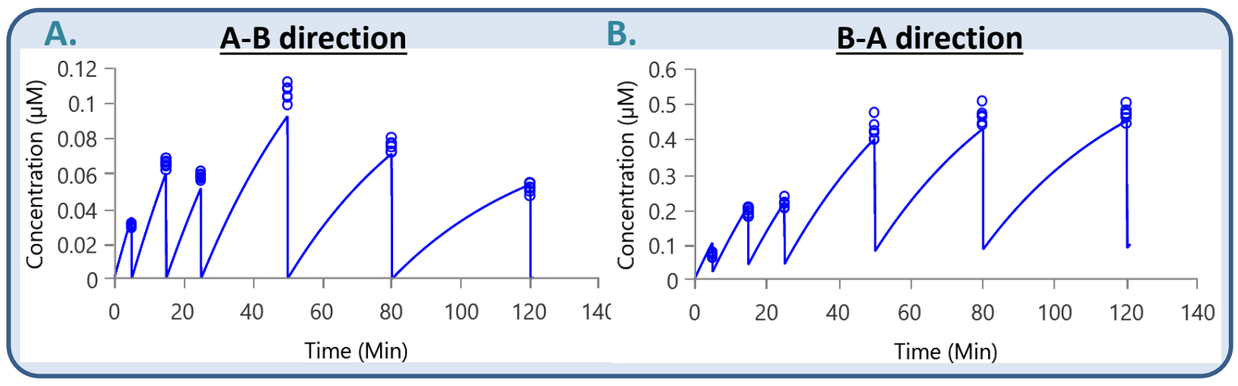

Figure 2. Metoprolol receiver well concentrations in bidirectional experiments conducted at a donor concentration of 1.1 µM (points) and the results of fitting the 3 compartment model in SIVA (lines).

Sandwich cultured hepatocyte (SCH) studies

The uptake and efflux of rosuvastatin was determined in Sandwich Cultured Human Hepatocytes (SCHH) using a two-phase protocol[7,8]. Accumulation in cell lysate was determined during an 'uptake phase' with 1 µM rosuvastatin in media, under conditions in which bile canaliculi were intact and disrupted (i.e. +/- Ca^{2+} in media). Following a media wash-step, the disappearance of rosuvastatin from cell lysate and appearance in media was determined during a subsequent 'efflux phase' under both bile canaliculi conditions.

Using the SCH model within SIVA, all data were simultaneously analysed in order to obtain an intrinsic clearance (CLu_{int}) estimates:

Parameter	Units	Estimate	95% CI
Sinusoidal Uptake CLu_{int}	µL/min/10^6 cells	6.08	5.75 – 6.40
Sinusoidal Efflux CLu_{int}	µL/min/10^6 cells	0.127	0.085- 0.168
Canalicular Efflux CLu_{int}	µL/min/10^6 cells	0.252	0.193 – 0.311

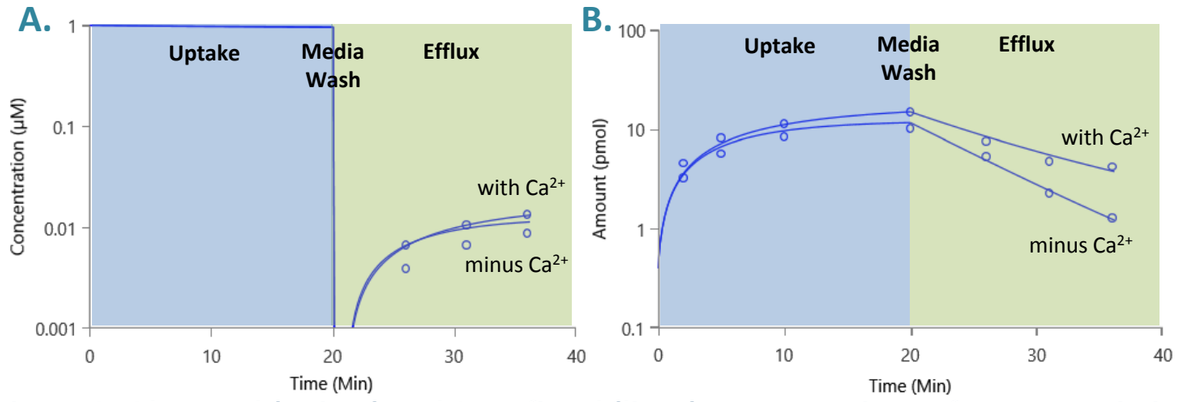

Figure 3. Observed (points) and SIVA fitted (lines) concentrations of rosuvastatin in media (A) and amounts of rosuvastatin in SCHH cell lysate (B).

Conclusion and Outlook

The SIVA 3 permeability/transport module includes:

- Accounting for sampling/replacement in 3 and 5 compartment models intended for the analysis of bidirectional transport (Transwell) studies.
- The Electrochemical Gradient Driven (EGD) transport model for the analysis of uptake experiments with electrogenic transporters such as OCTs, in addition to conventional uptake kinetics.
- Sandwich-cultured hepatocyte model with the ability to account for the application of inhibitors and media changes / wash steps.
- Confidence intervals and statistical evaluations.

Next, SIVA 4: MechP model for determining $P_{Trans,0}$, P_{para}, UWL in Transwell® assays.

References

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