Tracking Compounds and Their Interactions within In Silico Liver

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Abstract

We present multi-scale tracking features for the In Silico Liver (ISL) and their simulation results. The features were developed to support tracking of dynamic pharmacokinetic/pharmacodynamic (PK/PD) hepatic disposition phenomena that occur within an ISL during and after injection. Trackable components include molecules, enzymes, binders, cells, and features of tissue microarchitecture. Tracking has been performed across several levels of biological resolution including lobule, sinusoidal network, sinusoidal segments, cells, and binders. By tracking compounds and their spatiotemporal interactions, we could observe and analyze the reactive PK/PD responses and proactive hepatic events at the system level. Exploratory simulation experiments using ISLs in their normal and pathological states are expected to enable unraveling of the sophisticated biological realities that occur inside the liver at various scales.

1 Introduction

A mammalian liver consists of several lobes. Each lobe is a collection of many polyhedral lobules. A lobule is the primary hepatic unit involved in drug disposition and metabolism. It is comprised of portal vein tracts (PV), a central hepatic vein (CV), bile ducts, and an interconnected network of sinusoids. Hepatic blood flows from the PV to the CV through the sinusoidal network. Each sinusoidal segment (SS) is lined with endothelial cells that are separated from plates of hepatocytes and the perisinusoidal space.

In silico livers (ISLs) are in silico representations of a mammalian liver [1][2] in an experimental context. An ISL is a physiologically based, discrete-event driven, multi-agent based, synthetic simulation model. It represents multifaceted and heterogeneous aspects of hepatic anatomy and physiology. ISLs have been developed using discrete-event driven, multi-agent based modeling and simulation (M&S) techniques implemented on a high performance computing infrastructure [3]. It has been successfully applied to large-scale experimentation and analysis of hepatic disposition and metabolism.

ISLs have been validated [4] against in situ hepatic disposition profiles [5] of five different compounds, antipyrine, atenolol, labetalol, diltiazem, and sucrose. Each dose of simulated drug was administrated alone or in combination with sucrose or one of the other drugs. ISLs produced outflow profiles that were indistinguishable from the referent in situ profile data [5][6].

Multi-scale tracking features are being developed to track dynamic PK/PD phenomena that occur inside an ISL during and after dosing. They enable us to track compounds interacting with ISL components at all scales. Trackable components include drug molecules, enzymes, binders, cells, and features of tissue microarchitecture. Tracking is performed across multiple levels of biological resolutions, including lobule, sinusoidal network, and sinusoidal segment.

In this paper, we introduce multi-scale tracking features in the context of simulation results. It is expected that they will be used to answer questions that are difficult or currently infeasible to address using wet-lab PK/PD experiments.

2 Multi-scale traceability

Multi-scale traceability of the detailed dynamics within an ISL has been developed to achieve better observation and understanding of the spatiotemporal PK/PD activities thought to occur across multiple biological scales (Figure 1). The levels are lobule (L-level), sinusoidal network (N-level), hepatic zone (Z-level), sinusoidal segment (S-level), and cell (C-level). A zone is a partition of the sinusoidal network. Current ISLs have three zones [1]. A set of quantitative measures is used to track simulated drugs and spatiotemporal changes of ISL components. The measures include solute transit time (STT), solute travel path (STP), and temporal change of sinusoidal seg-

ment (TCS). STTs of simulated drugs are tracked at the five levels illustrated in Figure 1: L-level, Z-level, N-level, S-level, and C-level. STPs of the compounds are tracked at S-level, N-level, and C-level. TCSs of segments are tracked at N-level, S-level, and C-level.

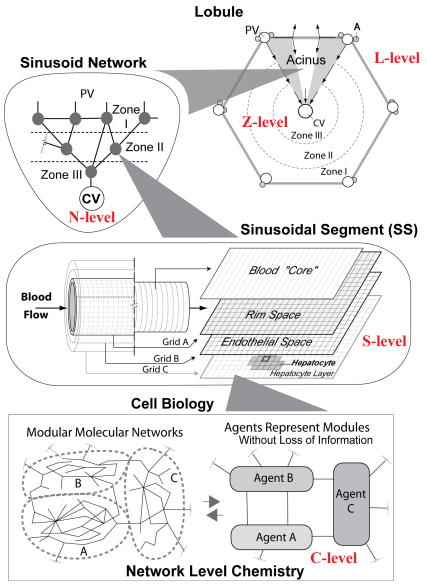


Figure 1: In silico liver: a lobule is comprised of several acini. A acinus consists of a portal vein (PV), a central vein (CV), and an interconnected network of sinusoids. Hepatic blood flows from the PV to the CV through the sinusoidal network. The network is divided into three zones. Both intra- and inter-zonal connections are possible. There exists three inter-zonal connections: Zone I → Zone II, Zone I → Zone III, and Zone II → Zone III. Each zone has at least one sinusoidal segment (SS). A sinusoidal segment contains a core and three grid spaces: sinusoidal space (Grid A), endothelial space (Grid B), and perisinusoidal space (including hepatocytes) (Grid C). Grid A, B, and C are two dimensional toroidal spaces that represent sinusoidal layer near endothelial cells, the endothelial layer, and the space of Disse and hepatocytes, respectively. In silico endothelial cells, hepatocytes, drug binders, and enzymes are added to these spaces.

In silico conditions were specified using a ISL parameter file and a lobule specification (Appendix A and B). The parameter file contains the parameters and values used to specify ISL structural and physiological properties, drug properties, and experimental protocols. For execution and analysis, experiments were performed on an in-house, eight-node, Linux beowulf cluster machine using Swarm, MPI, and R [7][8][9]. As in [1], for quantitative control of simulated drug input, we used the following dosage function:

$$d(t) = a * \frac{b^c * t^{c-1} * e^{-b*t}}{(c-1)!} \quad (1)$$

a, b, and c are three parameters that determine the location and shape of the function. t is time and e is an exponential function. 1000, 1, and 2 were used for a, b, and c, respectively. Results of tracked, simulated antipyrine are presented in the following sections.

3 Solute transit time trace

Solute transit times (STTs) of simulated drugs are tracked at L-level, N-level, Z-level, S-level, and C-level. The STT of each extends from its initial injection to and its arrival at the CV. Time is measured in seconds.

The mean STT at L-level was 22.9 (Figure 2). The mean STTs at Z-level were 15.1 for Zone I, 5.8 for Zone II, and 0.7 for Zone III. Residence in Zone I accounted for much of STT primarily because the available space in Zone I is larger than in the other two zones. The available space can be indirectly controlled by changing the number of nodes in each zone (Appendix B).

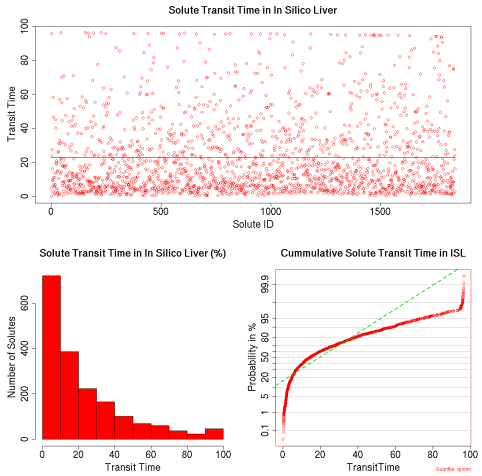
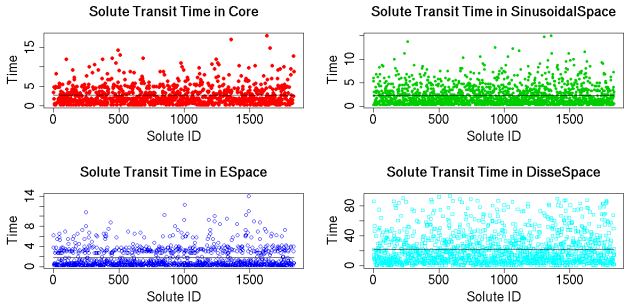


Figure 2: Solute transit time at L-level: The top panel shows STT of the simulated drug. The solid line is the mean STT of all compounds. The bottom two panels illustrate the same STT using a histogram plot and a probability plot. The dotted line in the probability plot is the line passing through the quartiles. The simulation terminated 95 second after dosing. The bar at the right side of the histogram indicates compounds that have not yet exited. Their STT was not determined.

The mean STT at the S-level was 2.7 for the blood core, 2.2 for the sinusoidal space, 1.7 for the endothe-

lial space, and 22.2 for the perisinusoidal space, respectively (Figure 3a). The number of simulated compounds in the perisinusoidal space was significantly reduced when the compounds did not penetrate membranes. Membrane permeability was controlled by the parameter *membraneCrossing* (Figure 3b). When the parameter was set to *NO*, the average STTs were changed to 10.7 for the core, 6.8 for the sinusoidal space, 1.2 for the endothelial space, and 1.6 for the perisinusoidal space.

a. *membraneCrossing* is YES



b. *membraneCrossing* is NO

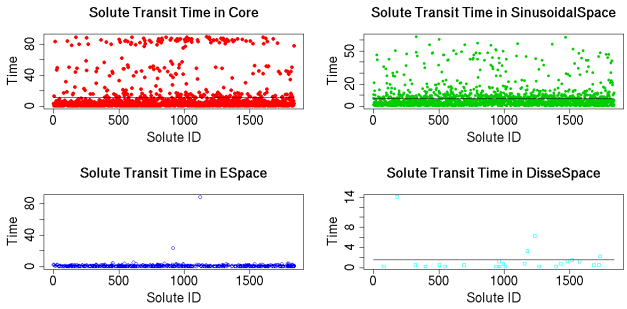


Figure 3: Solute transit time at S-level: when (a) membrane permeation was allowed and (b) membrane permeation was disabled. The parameter *membraneCrossing* significantly influenced the number of simulated compounds and their binding to cellular components in the perisinusoidal space. The solid line in each graph represents the mean STT of all components.

The mean STT at C-level was 0.6 for endothelial cells, 7.7 for hepatocytes, 3.5 for binders, and 15.9 for enzymes (Figure 4). STTs at C-level represent cumulative binding time to cellular components in the three spaces. However, STTs of simulated drug within hepatocytes, binders, and enzymes were not tracked because they do not exist in the perisinusoidal space when *mebraneCrossing* is set to *NO*. Binding frequency was indirectly controlled by a set of ISL parameters including, *ECDensity*, *HepDensity*, *SoluteBindingProb*, *SoluteBindingCycles*, *BinderPerCellMin*, *S2EJumpProb*, *E2SJumpProb*, *E2DJumpProb*, and *D2EJumpProb*. For more detail, see [1].

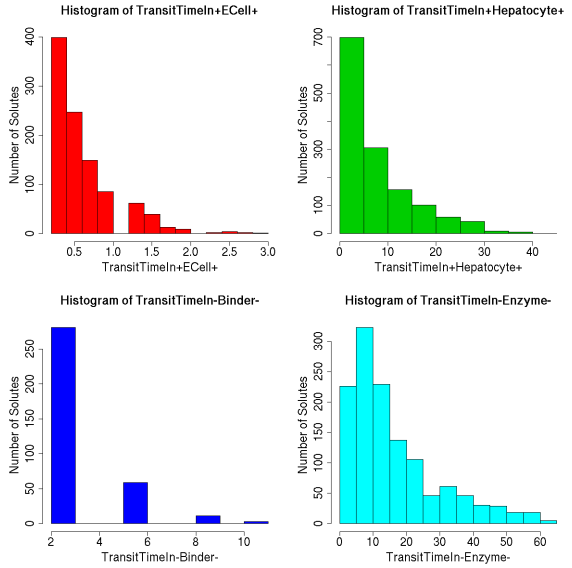


Figure 4: Solute transit time at C-Level; STTs of simulated drug compounds bound to in silico endothelial cells(top left panel): to in silico hepatocytes (top right); to in silico binders(bottom left); and to in silico enzymes (bottom right).

4 Solute travel path trace

Solute travel paths (STPs) were tracked at N-level, S-level, and C-level. The average solute travel path length was 17.9 (Figure 5).

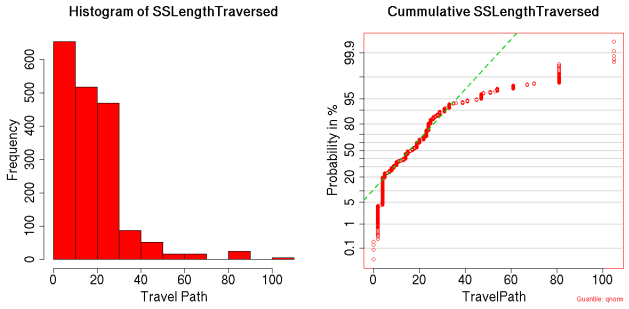


Figure 5: Solute travel path: (a) the histogram of solute travel paths of injected simulated drugs (left panel) and (b) the probability plot of the paths (right panel).

From initial injection until final collection, simulated drug was bound to all or part of in silico endothelial cells, hepatocytes, binders, and enzymes.

The average binding time for these cellular components were 1,4, 23.0, 2.7 and 44.1, respectively. The overall binding time was indirectly controlled by changing these ISL parameters: *SoluteBindingProb*, *EnzymeInductionRate*, *EnzymeInductionProb*, and *MetabolizationProb*. For more detail, see [1].

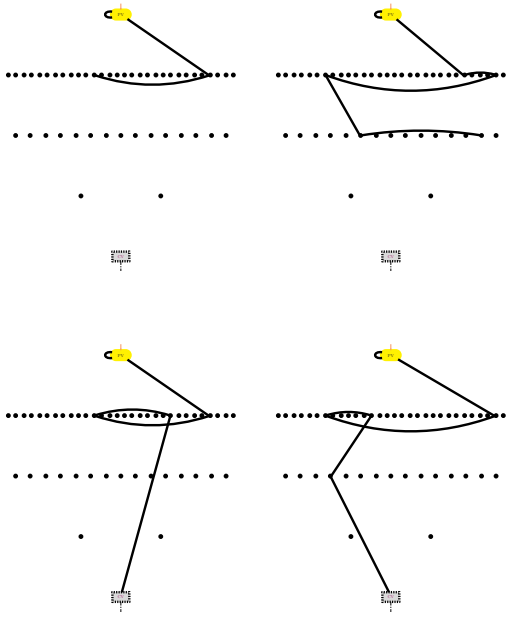


Figure 6: Four solute travel paths from start until the end of the simulation, selected at random: PV and CV are top and bottom terminal nodes of each panel. Zone I, II, and III are configured by 30, 15, and 2 SS. The travel path of a simulated drug is a directed line that starts from PV and ends at some SS node when the simulation ends or when the drug object reaches CV.

5 Temporal change of sinusoidal segments

Temporal changes of sinusoidal segments (TCSs) were tracked at N-level, S-level, and C-level. Temporal change of the drug bolus injection/disposition ratio was determined by tracking temporal changes of PV and CV. The temporal changes in the number of endothelial cells, hepatocytes, enzymes, and binders that are bound to drug compounds are tracked at C-level (Figure 7).

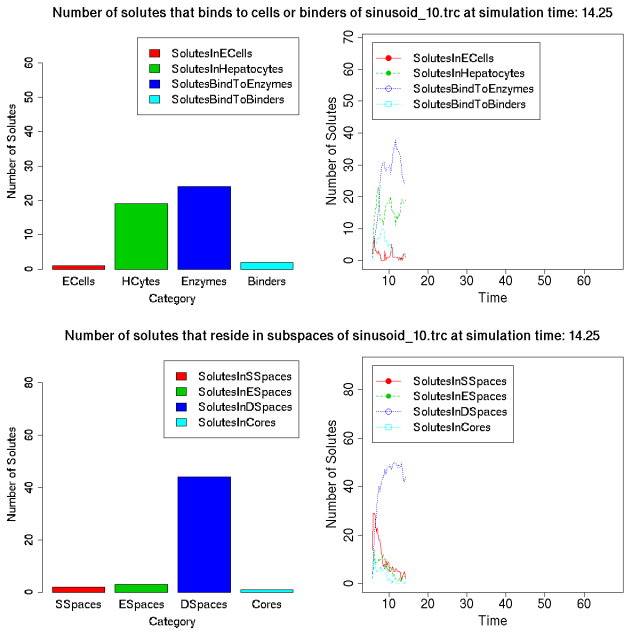


Figure 7: Temporal change of a sinusoidal segment: left panels show the number of simulated compounds bound by different cellular components at a specified time. Panels on the right show temporal changes to the numbers.

6 Conclusion

We developed multi-scale tracking features for ISLs to provide better observation, analysis, and understanding of complex PK/PD phenomena that occur within ISLs. It has been applied to hepatic drug disposition and metabolism of cationic drugs. It is expected to become an effective in silico method for PK/PD and toxicity research.

Acknowledgements

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Appendix

A In silico liver parameters

ISL parameters are divided into four groups: simulation execution and evaluation (SEE), articulated synthetic model (ASM), dosage parameter and time (DPT), and referent mathematical model (RMM). See [1] for more detailed information on each parameter.

Parameters in the SEE group provided information required to execute and evaluate an in silico experiment (Table 1). *monteCarloRuns* represents the number of simulation runs executed during the experiment. A single simulation run requires *cycleLimit* simulation iterations. A simulation iteration is compound of *stepsPerCycle* simulation cycles. The total number of simulation cycles executed during the experiment is *monteCarloRuns* × *cycleLimit* × *stepsPerCycle*. Experimental results are evaluated using *similarityMeasure*. *similarityMeasure* is a quantitative method that computes similarity between simulated profiles of ISL and referent in situ profiles.

Table 1: Simulation execution, monitor, and evaluation parameters

Parameter	Value	Data Type
monteCarloRuns	2	NUMERIC
cycleLimit	200	NUMERIC
stepsPerCycle	2	NUMERIC
currentRuns	0	NUMERIC
similarityMeasure	global_sd	STRING
runFileNameBase	run	STRING
nominalProfile	dat	STRING
experimentalProfile		STRING

Parameters in the ASM group provided information on structural and behavioral properties of ISL (Table 2). *GraphSpecFile* specifies the file name that is used to create the sinusoidal network of the articulated synthetic model (See also Appendix B). There are two types of sinusoidal segments – direct and tortuous. *DirSinRatio* and *TortSinRatio* specify the ratio of these segments in the network. The circumference and the length of each segment are computed based on *DirSinCircMin*, *DirSinCircMax*, *TortSinCircMin*, *TortSinCircMax*, *DirSinLenAlpha*, *DirSinLenBeta*, *DirSinLenShift*, *TortSinLenAlpha*, *TortSinLenBeta*, and *TortSinLenShift*.

Drug compound movement between spaces is controlled by *S2EJumpProb*, *E2SJumpProb*, *E2DJumpProb*, and *D2EJumpProb* in a probabilistic manner. The speed of hepatic blood stream and local turbulence in the stream are controlled by *CoreFlowRate* and *SinusoidTurbo*. Population of endothelial cells and hepatocytes is presented by *ECDensity* and *HepDensity* in terms of the density per grid space. The numbers of binders and enzymes, and their binding time and probability are specified by *BindersPerCellMin*, *BindersPerCellMax*, *EnzymeInducWindow*, *EnzymeInducThreshold*, and *EnzymeInducRate*. Bindings between drug compounds and ISL cellular components are governed by *SoluteBindingProb* and *SoluteBindingCycles*. Drug metabolism is determined by *MetabolizationProb*. The mapping between an in silico compound and an in vivo compound is specified by *SoluteScale*.

Table 2: Sinusoidal network structure parameters

Parameter	Value	Number
GraphInputFile		STRING
GraphSpecFile	lobule	STRING
GraphSpecIterates	1	NUMERIC
DirSinRatio	0.90	NUMERIC
TortSinRatio	0.10	NUMERIC
DirSinCircMin	*50*	NUMERIC
DirSinCircMax	50	NUMERIC
DirSinLenAlpha	2.0	NUMERIC
DirSinLenBeta	0.215	NUMERIC
DirSinLenShift	0.0	NUMERIC
TortSinCircMin	4	NUMERIC
TortSinCircMax	4	NUMERIC
TortSinLenAlpha	10.0	NUMERIC
TortSinLenBeta	0.10	NUMERIC
TortSinLenShift	-35.0	NUMERIC

Table 3: Spatiotemporal activity parameters

Parameter	Value	Number
S2EJumpProb	0.5	NUMERIC
E2SJumpProb	0.5	NUMERIC
E2DJumpProb	0.2	NUMERIC
D2EJumpProb	0.5	NUMERIC
CoreFlowRate	3	NUMERIC
SinusoidTurbo	0.1	NUMERIC
ECDensity	0.95	NUMERIC
HepDensity	0.95	NUMERIC
BindersPerCellMin	5	NUMERIC
BindersPerCellMax	37	NUMERIC
EnzymeInducThreshold	1	NUMERIC
EnzymeInducRate	0.5	NUMERIC
EnzymeInducWindow	10	NUMERIC
MetabolizationProb	0.1	NUMERIC
SoluteBindingProb	0.15	NUMERIC
SoluteBindingCycles	10	NUMERIC
SoluteScale	7.0	NUMERIC

Table 4: Dosage function and time parameters

Parameter	Value	Data Type
Drug	Antipyrine	STRING
MembraneCrossing	TRUE	STRING
a	1000	NUMERIC
b	1	NUMERIC
c	2	NUMERIC
start	5	NUMERIC
end	70	NUMERIC

Parameters in the DPT group provided information on simulated drug type, dosage function, and dosing time (Table 4). Simulated Antipyrine drug compounds are injected into ISL using the dosage function described by equation (1) for the start and the end of drug bolus injection. Cell membrane permeability of the simulated drug compound is controlled by *MembraneCrossing*.

Parameters in RMM group provide information on the referent mathematical model (Table 5). The model represents hepatic disposition of isolated perfused rat liver using a set of PK/PD parameters, stochastic processes, and inverse Laplace transform [5].

Table 5: Referent mathematical model parameters

Parameter	Value	Data Type
TimeStart	7.0	NUMERIC
TimeIncrement	0.1	NUMERIC
k1	0.03	NUMERIC
k2	0.01	NUMERIC
ke	0.1	NUMERIC
DispersionNum	0.26	NUMERIC
ExpTransitTime	6.35	NUMERIC
BolusMass	1.0	NUMERIC
PerfusateFlow	0.312	NUMERIC
MainDivertRatio	0.00654	NUMERIC
SecDivertRatio	0.0248	NUMERIC
Epsilion	10^{-24}	NUMERIC
datInterpolate	true	STRING
simularityBandCoe	1.0	NUMERIC
contractRatio	0.5	NUMERIC
reflectionRatio	1.5	NUMERIC
expandRatio	1.8	NUMERIC
shrinkRatio	0.5	NUMERIC

B Lobule specification

Lobule specification is used to describe the structural topology of the sinusoidal network within the lobule. It divides into two parts: the number of nodes for each zone and the number of edges between two zones. The network is randomly created based on this specification. The following specification is used for all experiments.

nodes per zone
30 15 2
edges
15 15 0
0 7 2
0 0 0