# Falsification/Validation of Enzyme Induction Mechanisms within a Validated, Multiscale Liver Model

**C. Anthony Hunt, Glen E.P. Ropella,** and **Ryan C. Kennedy**

Bioengineering and Therapeutic Sciences, University of California, San Francisco, CA

## Introduction

The In Silico Liver (ISL) is part of a proof of principle project demonstrating exploratory experimental methods on synthetic, computational analogs. We explore concrete hypotheses for potential wet-lab experiments. At some scales and in some aspects, the liver is well behaved and well characterized. At other scales and in other aspects, livers can be complex without any feasible modeling methods. Aspect is defined as the perspective taken when an analog is observed (including the phenomena on which we focus). Agent-based modeling was chosen as the base platform because it facilitates multi-models across all spectra: homogenous to heterogeneous, continuous to discrete, shallow to deep, regular to chaotic, and across all representation paradigms including equation-based, rule-based, cellular, lattice-free, etc.

## Use Case

The target of this work in progress is hepatocytes' ability to regulate (up or down) their enzymes in response to encountered compounds and other physiological signals [1-3]. Results from a study on the in situ clearance of cationic drugs [4] provide the coarse-grained validation aspect on which we focus: the fraction of compound exiting in relation to the amount of compound in the bolus. Validation of various ISLs against data from the cited experiments is presented in these previous reports [5-7].

The aspect and measure investigated here is enzyme count in each zone following each simulation cycle. Because of the way the mechanism was constructed, current enzyme count is a function of past compound and enzyme counts, and the metabolic events in each hepatocyte. Hepatic validation data is from in vivo experiments as opposed to in situ experiments. Because the ISL is composed of discrete software objects, it allows (indeed facilitates) individual measures to be similar to those used by the wet-lab experiments and it allows the composition of those measures. The composition of the mechanism produces an articulated structure where the inputs and outputs of each component can be traced and measured in a variety of ways that mimic wet-lab measures. ISL measures are kept programmatically separate and are applied as part of the experimental procedure, again mimicking the experimental wet-lab methods.

Another way to view the protocol is that each component, composite or atomic, presents its phenotype to the other components with which it is composed. So doing allows the hierarchical generation of coarse-grained, emergent phenomena from fine-grained emergent phenomena. The structure keeps the hierarchy explicit, which is necessary to mitigate the fact that the validation data is from entirely different experimental subjects, protocols, and use cases. Maintaining, curating, and reasoning about measure and aspect composition, over and above mechanism composition is a primary purpose behind in silico methods.

## Approach

This enzyme induction (EI) project follows the work in [5-7,11,15]. We focus on common patterns of EI but not specific drug metabolizing enzymes. The coarse-grained output profile is refined into measures for finer-grained phenomena exhibited by ISL components. It is currently infeasible to take multiscale data directly from a particular referent. The process demonstrated here of composing the coarse-grained measures with finer-grained ones, allows us to shrink the set of plausible fine grain mechanisms. Because hepatocytes play central liver function roles, establishing the autonomy of hepatocytes used in the ISL is critical.

Fine-grained tracing measures, such as those used in [5-7] are useful for selecting a subset of plausible, concrete hypothetical mechanisms. However, they are not driven by fine-grained quantitative validation data taken directly from experiments on livers. The 2D liver lobule cross-section in [11] takes validation data from the literature and constructs a model where measures of simulation details are quantitatively similar to wet-lab counterparts. The primary distinction between the 2D zonation analog and the ISL lies in the irregularity of the lobule graph topology. This work strives to achieve a more concrete EI mechanism than that of the 2D zonation analog in [11]. Here, we use a hybrid (combined continuous and discrete) mechanism using the discretized continuous equations for R- and B-signals from [11].

## Methods

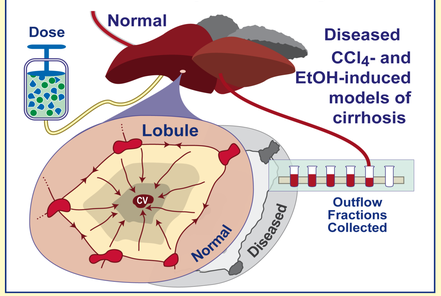
ISL components and features described in detail in [5] are illustrated in the adjacent figures. Hepatocytes contain [0-100] enzymes, which metabolize bound compound each iteration if a uniform pseudo-random draw is less than the number of enzymes divided by 100.

### *Enzyme Induction*

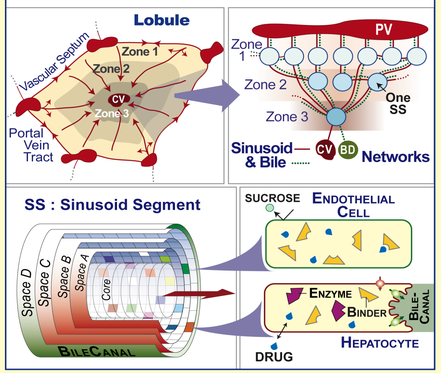
Within each hepatocyte, enzymes are induced and eliminated as a function of amounts of compound and enzymes present some number of iterations in the past, specified by the induction queue (|IQ|tgt) size. Further, enzymes are also eliminated as a function of the hepatocyte's distance from the PV, according to a gradient that increases along the maximal path length from PV to CV. Figure 1 is a visual description of the mechanism. Below is a precise description of how the mechanism works.

The push() and pop() functions indicate value insertion onto the back and removal from the front of the queue, respectively. For example, when the size of the induction queue is zero, zero is pushed onto the back of the queue. When the queue size is larger than it should be, nothing is added and the value in front is removed. The EI mechanism relies on compound being detected within an hepatocyte, regardless of a metabolic event. Induction is based on the number of compound objects inside a hepatocyte.

## The Wet-Lab Experimental System




## In Silico Liver (ISL) Components




### *ISL's Multiple Scales*

ISL's generator-to-phenomena map consists of four integration types: 1) measures, 2) sequencing, 3) frequency, and 4) mechanism. This paper focuses on measure integration across scales. There are three sequencing types: discrete event (DE), discrete time (DT), and DT implemented as periodic DE. Intracellular solute is bound stochastically and release is scheduled as a DE. All other DEs are scheduled periodically with a given frequency. The experiment agent and some measures operate at lower frequencies. The EI equations are computed in terms of the current cycle interpreted as discretized continuous time. Mechanisms are composed to span seven scales: objects that map to molecules (binders, enzymes, and solute), cells, intra-sinusoidal spaces, sinusoidal segments (SS) and their connections, the lobule graph, the three model agents, and the experiment agent. The lobule component of the ISL only spans five scales. The model and experiment agents are required to fully represent the experimental protocol (use case).

### *Coarse Grain Results*

The coarse-grained outflow profiles shown validate the same, as did previous ISL outflow profiles. ISL phenotype was explored automatically with coarse parameter sweeps in all metabolism and induction parameters.

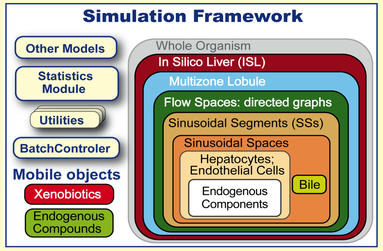
### *Extended Execution*

The EI validation data is from a model protocol where rats were sacrificed 12 hours and 3 days after exposure [13-14]. This point is not thought to be critical because we expect zonation can occur quickly in response to a bolus of an appropriate inducing compound. However, it might be relevant to the transients of the EI mechanism, the environment of the discrete lobule, or the hybrid integration between them.

### *Recirculation and additional endocrine signals*

Related to the above, longer execution, the animal models used for most zonation studies also include fluid (blood) recirculation and multiple up and down regulatory signals for different enzymes. For example, Oinonen and Lindros [12] show the involvement of pituitary and gonadal hormones. Consequently, having perfusate recirculate over longer execution times should change the dynamics of induction. Because elimination is a delayed function of the enzymes present, it is possible that zonation would arise as concentration of the compound in solution decreased. Adding more regulatory signals to make the mechanism more accurate would increase the ISL control surface and make a) validation easier and b) the model even less falsifiable. However, neither of these results would satisfy the objectives of the project.

## Simulation Framework




## EI Mechanism

We falsified three, somewhat simpler EI mechanisms [6-7]. To decouple induction from metabolism, we designed hepatocyte EI to trigger the creation of new enzymes based on the number of compound object present in the hepatocyte. The scheduler handles about 2 million events per model iteration. The EI mechanism dynamically, illustrated in the adjacent figure, creates and destroys enzyme objects, which increases and decreases the number of events in any given cycle. Although the ISL scheduler forcibly maintains memory allocation so that it grows and shrinks with the allocation of memory, such dynamism adds both memory and computational overhead. In high induction experiments, the EI mechanism caused memory faults for long execution times. To avoid that problem, we removed the individual schedules contained in each cell object and changed their induction and elimination mode from discrete event (DE) to discrete time (DT). More specifically, hepatocytes step through the same logic each iteration, creating or destroying enzymes according to a data structure they maintain, rather than placing induction or elimination events on the schedule. Construction of metabolite and destruction of compound remains DE. From a modeling perspective, the pseudo-randomized ordering of hepatocyte execution mitigates the potential sequencing artifact, which may be introduced. In addition, the ExperAgent, which executes and monitors the simulation, maintains data structures of observations for every iteration of the simulation. For long experiments, that process can use considerable memory. To manage memory efficiently, a parameter was added that disables that process; the derived measures used for verification and validation are calculated off-line after execution is complete. These two technical changes allowed executing experiments over 86,400 model iterations. Larger numbers are feasible. So doing allows a fully fine-grained ISL to span a 24-hour period at one iteration per second of simulated time.

The ISL has been ported to the MASON simulation toolkit. The EI mechanism uses a gradient (decreasing from PV to CV) as in [11]. It decreases exponentially from 1.0 at the perivenous region to near 0.0 in the centrilobular region. It represents resources made available to the hepatocytes (and other cells) by blood flow. EI is a function of the gradient. EI uses the same scheduling mechanism for induction and elimination.

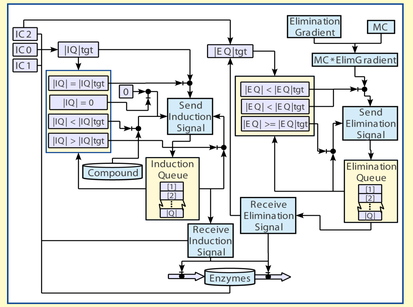
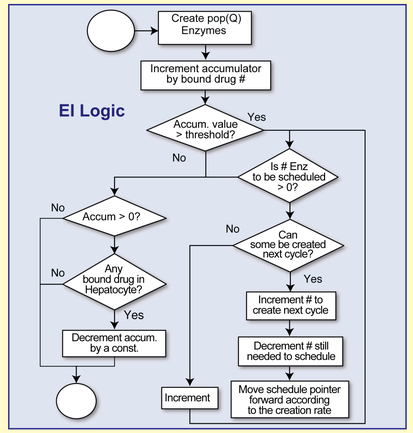


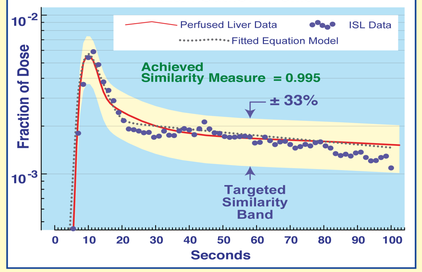

**Figure 1.** Diagram of the implemented EI mechanism. IC[0-2] are the induction constant parameters. Arrows indicate influence, positive or negative. Valves are turned on or off by their controller inputs where turning them on allows data to flow. IC[0-2], MC, and 0 are constants. Drug and enzymes are "stocks" that increase or decrease over time (the increase or decrease of compound is not controlled by the EI mechanism). The left side of the diagram represents induction and the right side indicates elimination, as indicated by flow control into and out of the enzyme stock. Walking through the induction sub-graph, IC[0-2] parameterize the calculation of the induction queue (|IQ|tgt) size and the elimination queue (|EQ| tgt). The target size for the induction queue is used to calculate the value installed on the induction queue. E.g., if the queue is smaller than its target size, compound number is used for the induction signal. If the queue is too large, it simply takes the next value on the queue. If the queue is empty, there is no signal. When the queue has reached its target size, the induction equation is used. Note that the induction signal can be negative, allowing it to raise or lower the stock of enzymes. A similar reading applies for the elimination sub-graph on the right side. Queues advance once within an experimental model cycle, twice within an experiment agent cycle.




## EI Mechanism (cont.)

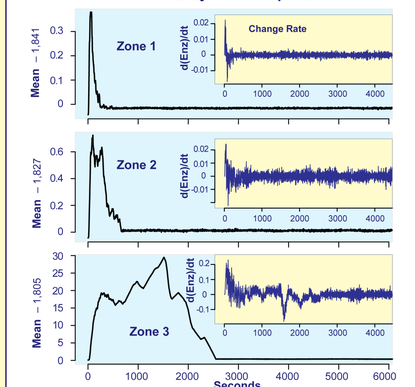
Each of the approximately 11,000 hepatocytes in any given trial (single execution) maintains two data structures, one for induction and one for elimination, containing the number of enzymes to induce or eliminate at each cycle. For some parameter values, induction or elimination is scheduled every step far in the future. So doing can lead to large queues and expanded memory use. In order to limit memory use, for cases when induction or elimination would lead to a queue larger than the number of enzymes already present, respective accumulators are incremented without actually scheduling the induction or elimination. Accumulator values are incremented each cycle and used to test against the thresholds beyond which the hepatocyte will try to schedule or induce enzymes. Such a protocol provides a "relaxation" window enabling an overly stimulated (to induce or eliminate) hepatocyte to continue in that state for some number of cycles after the stimulus is gone. Preliminary output data from 1100-second experiments are shown.

## Diltiazem Outflow Profiles

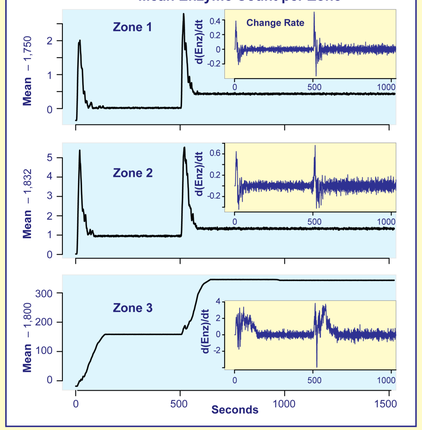



Coarse Grain Validation: The match between ISL, diltiazem, and sucrose outflow profiles achieved the prespecified Similarity Measure.

## Mean Enzyme Count per Zone




## Mean Enzyme Count per Zone




## References

1. Usynin IF, LE Panin. 2008. Mechanisms determining phenotypic heterogeneity of hepatocytes. Biochemistry (Mosc). 73(4):367-380.
2. Mankowski DC, S Ekins. 2003 Prediction of human drug metabolizing enzyme induction. Curr Drug Metab. 4(5):381-391.
3. Lerapetritou MG, PG Georgopoulos, CM Roth, LP Androulakis. 2009. Tissue-level modeling of xenobiotic metabolism in liver: An emerging tool for enabling clinical translational research. Clin Transl Sci. 2(3):228-237.
4. Hung DY, P Chang, K Cheung, B McWhinney, PP Masci, M Weiss, MS Roberts. 2002. Cationic drug pharmacokinetics in diseased livers determined by fibrosis index, hepatic protein content, microsomal activity, and nature of drug. J Pharmaco Exp Ther. 301(3):1079-1087.
5. Park S, GE Ropella, SH Kim, MS Roberts, CA Hunt. 2009. Computational strategies unravel and trace how liver disease changes hepatic drug disposition. J Pharmacol Exp Ther. 328(1):294-305.
6. Park S, SH Kim, GE Ropella, SH Kim, MS Roberts, CA Hunt. 2010. Tracing multiscale mechanisms of drug disposition in normal and diseased livers. J Pharmacol Exp Ther. 334(1):124-136.
7. Kim SHJ, S Park, GEP Ropella, CA Hunt. 2010. Agent-directed tracing of multi-scale drug disposition events within normal and diseased In Silico Livers. Int J Agent Technol Systems 2(3):1-19.
8. Sheikh-Bahaei S, CA Hunt. 2011. Enabling clearance predictions to emerge from in silico actions of quasi-autonomous hepatocyte components. Drug Metab Dispos. 39 (10):1910-1920.
9. Ropella GEP, S Park, CA Hunt. 2008. Evaluating an hepatic enzyme induction mechanism through coarse- and fine-grained measurements of an In Silico Liver. Complexity. 14(6):28-34.
10. Sheikh-Bahaei S, SH Kim, S Sheikhbahaei, CA Hunt. 2010 Understanding the role of liver zonation in toxin elimination. Int J Intel Control Syst. 14(1):33-40.
11. Sheikh-Bahaei S, JJ Maher, CA Hunt. 2010 Computational experiments reveal plausible mechanisms for changing patterns of hepatic zonation of xenobiotic clearance and hepatotoxicity. J Theor Biol. 265(4):718-733.
12. Oinonen T, KO Lindros. 1998. Zonation of hepatic cytochrome P-450 expression and regulation. Biochem J. 329(1):17–35.
13. Selim N, GD Branum, X Liu, R Whalen, TD Boyer. 2000. Differential lobular induction in rat liver of glutathione S-transferase A1/A2 by phenobarbital. Am J Physiol Gastrointest Liver Physiol. 278:G542–G550.
14. Santostefano MJ, VM Richardson, NJ Walker, J Blanton, KO Lindros, GW Lucier, SK Akasey, LS Birnbaum. 1999. Dose-dependent localization of TCDD in isolated centrilobular and periportal hepatocytes. Toxicol Sci. 52(1):9-19.
15. Ropella GEP, CA Hunt. 2012. Zonation of Drug-induced, Hepatic Enzyme Induction: Falsifying Fine Grained Mechanisms within a Validated, Multiscale In Silico Liver. Spring Simulation Multiconference 2012, Agent-Directed Simulation Symposium (ADS'12), The Society for Modeling and Simulation International, Orlando, FL.

## Acknowledgement

This work was supported in part by grant G10C20235 from the EPA (James Glazier, PI). Earlier results were reported in [15]

***Legend.*** These results show EI spread (zonation) over the entire lobule, an emergent property, following a single drug dose. Y-axis: # enzymes/# of SS nodes in that zone, approximating a measure of enzyme expression density in liver tissue. Zone III shows a much higher EI than zones I and II, reflecting a qualitative zonation pattern seen in wet-lab experiments. EI in all three zones drops to a low, stable state. Zones I and II do so quickly whereas the zone III returns to normal levels after 600 seconds. Data: average of 96 Monte Carlo trials. Relevant parameters: EI and drug elimination rates are 0.05, EI and EL responses are 0.25, gradient shape is 5.6.

***Legend.*** These results show EI spread over the entire lobule following two doses. Y-axis: same as above. Zone III shows a much higher EI than zones I and II, reflecting a qualitative zonation pattern seen in wet-lab experiments. EI in all three zones drops to a low, stable state. Zones I and II do so quickly whereas the zone III returns to normal levels after 600 seconds. Data: average of 96 Monte Carlo trials. Relevant parameters: EI and drug elimination rates are 0.05, EI and EL responses are 0.25, gradient shape is 5.6.

## Summary

The focus is an In Silico Liver (ISL) model family and an evolving suite of mechanistic hypotheses about (rat) liver-drug interactions. ISLs are multiscale and hierarchical. A medium grain Enzyme Induction (EI) mechanism was implemented. Validation (falsification) of complicated, knowledge-based models requires integrating distinct aspects and methods for multi-aspect validation. For ISLs, such integration has not been straightforward. Falsification is crucial for formulating, testing, and iteratively evolving hypotheses about liver mechanisms. During multi-aspect falsification we can falsify a hypothesis in one aspect (emergent EI) while simultaneously validating it in another aspect (drug disposition profile). We demonstrate a multi-scalar validation/falsification event in which we validate the mechanism against coarse grain measures of liver perfusate drug levels and falsify it against a medium grained measure of hepatic zonation. Falsification is guiding mechanism (hypothesis) refinement. The ability to scale validation efforts is necessary for effective scientific use models such as ISLs.