Simulating Microdosimetry of Environmental Chemicals for EPA's Virtual Liver

John Wambaugh and Imran Shah
National Center for Computational Toxicology
Office of Research and Development
U.S. Environmental Protection Agency
Research Triangle Park, NC

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The contents of this presentation do not necessarily reflect EPA policy.

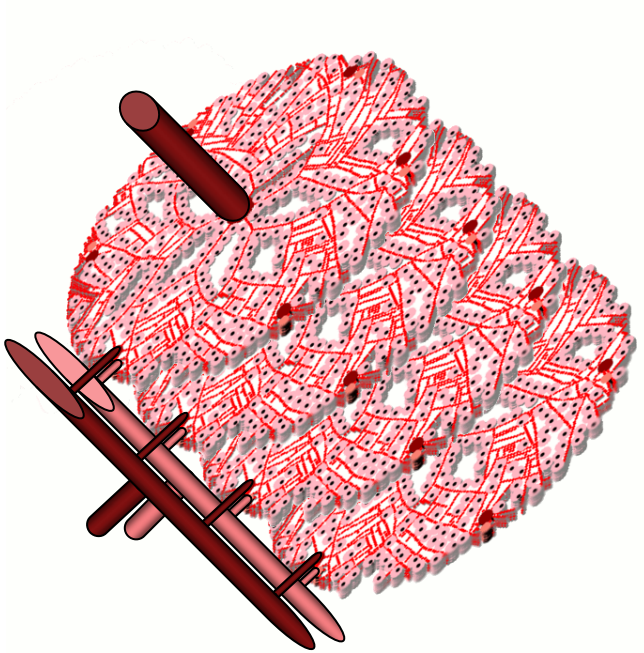
Introduction

More than 6000 compounds that could impact human health through the environment

The liver serves a primary function altering and eliminating chemicals from the blood stream

Rodent liver cancer is a common adverse effect used for determining acceptable levels

Developing a cellular systems model of hepatic tissues to provide simulated *in vivo* context (*in silico* or virtual) for *in vitro* data

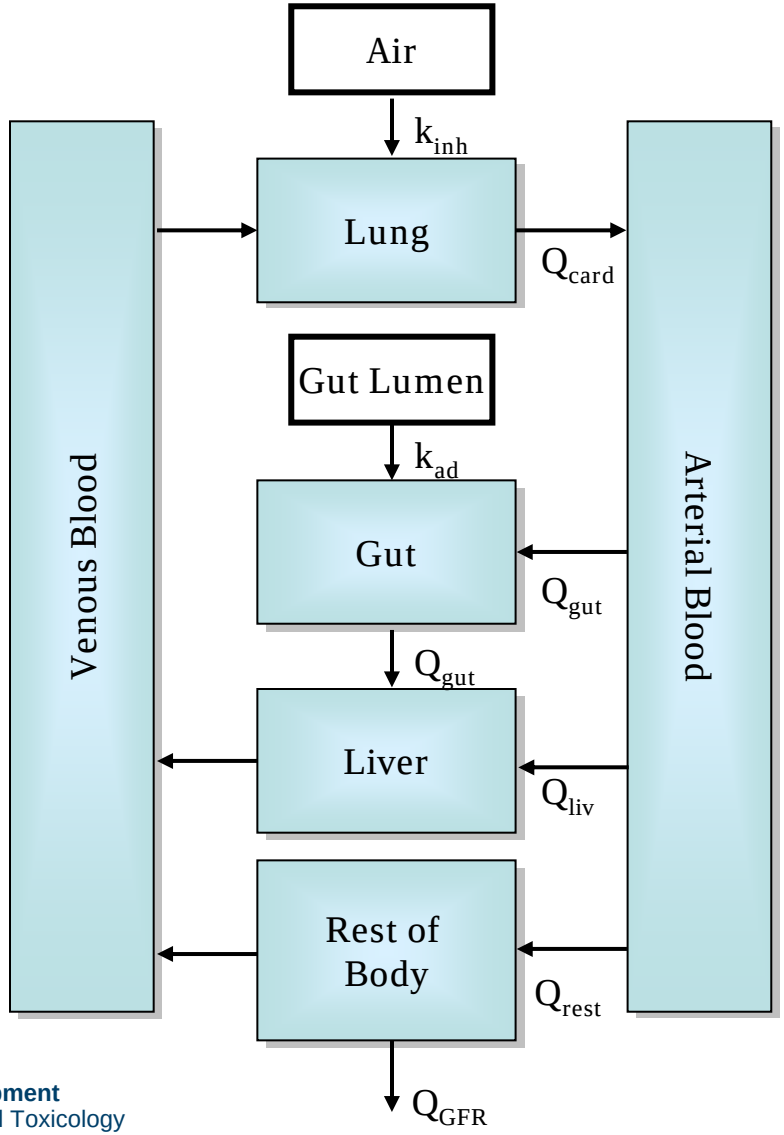


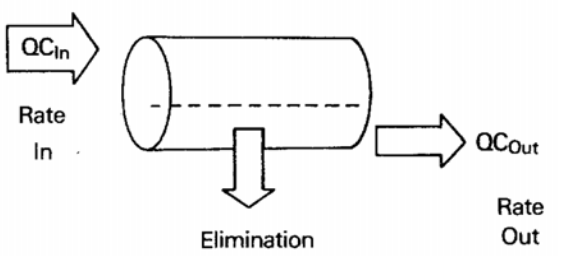
Focusing on simulating **dose**-response for a single “classic” lobule

Function of hepatocytes known to be **spatially heterogeneous** – agent-based approaches to tissue modeling assume that each cell’s behavior determined in reaction to its **local microenvironment**

Tissue Dosimetry from Pharmacokinetic Models

Traditional Physiologically-based Pharmacokinetic (PBPK) models treat tissues as being composed of a few **well-mixed** compartments

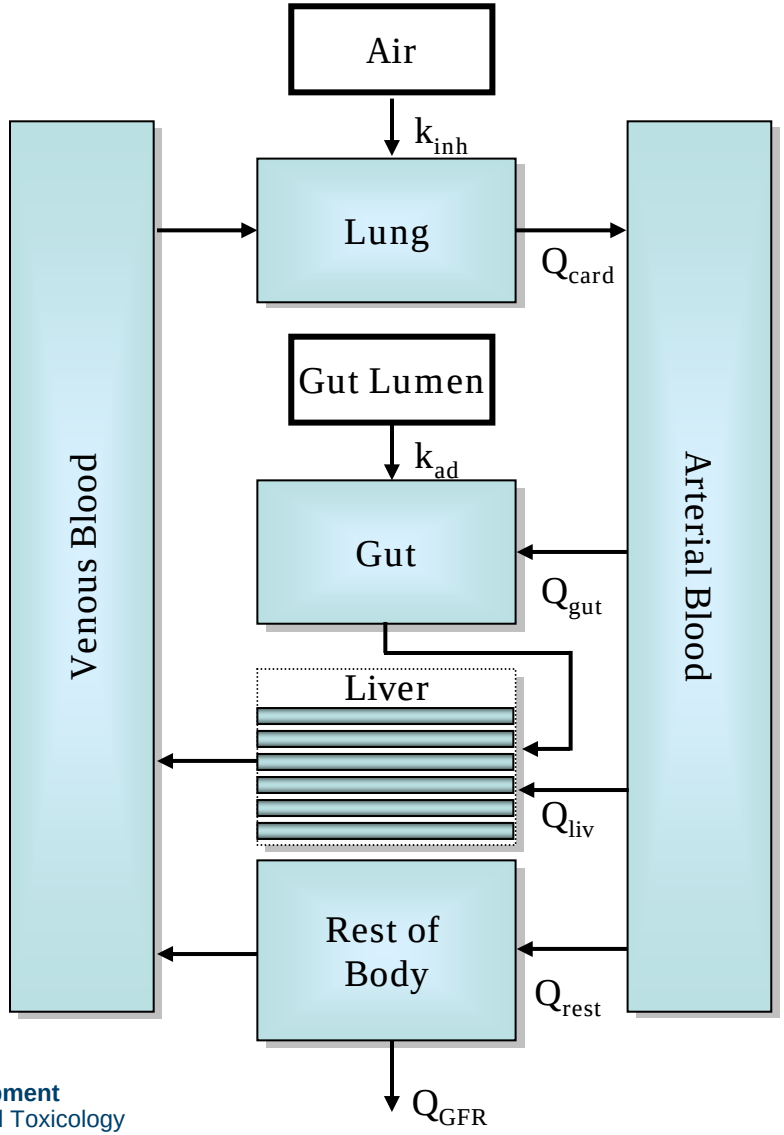


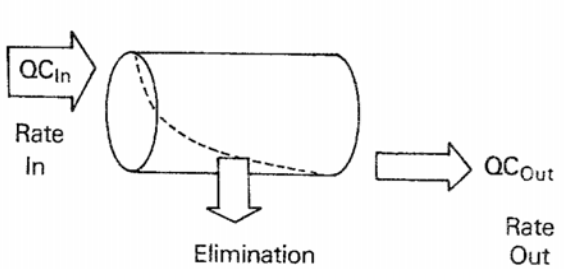


Pang, K. S. and M. Rowland (1977). J. of Pharmacokin. and Biopharm. **5**(6): 625-653.

Tissue Dosimetry from Pharmacokinetic Models

Depending on the compound, other approaches are sometimes used for the liver, such as many **parallel tubes** along which chemical concentration varies





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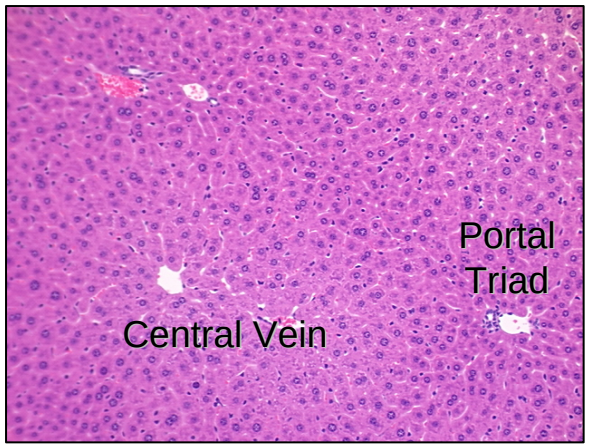
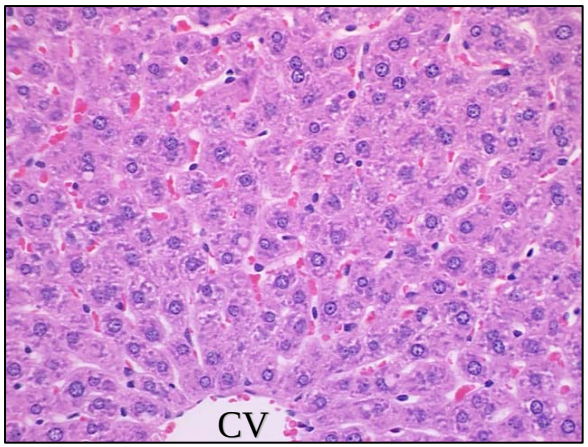
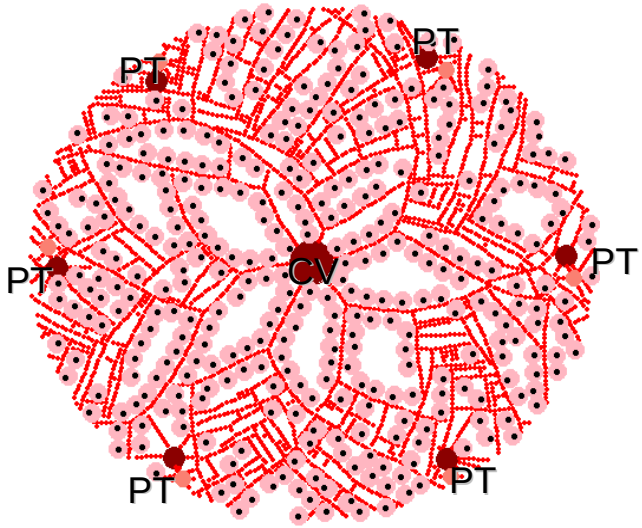
Dose-Response for a Virtual Lobule



The classic lobule consists of a single central vein fed venous and arterial blood via multiple portal triads



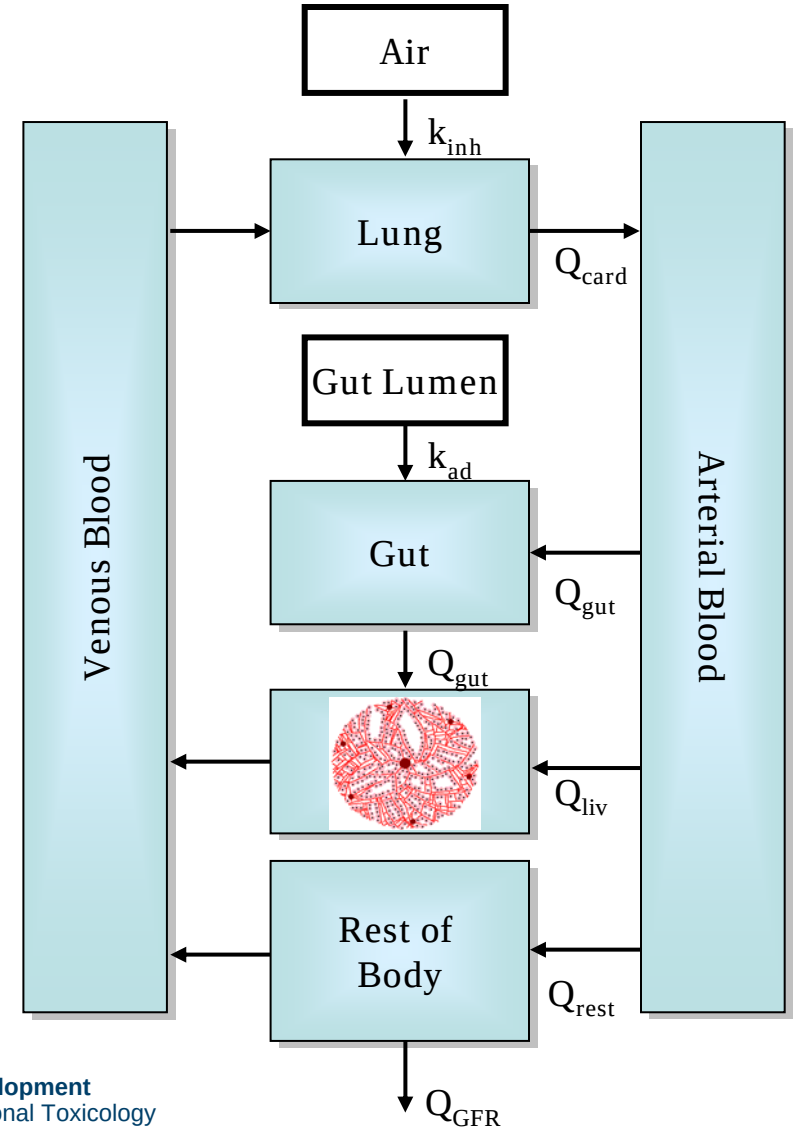
Blood flows through a network of sinusoids, supplying and exposing hepatocytes



Synthetic lobule sufficiently complex to determine that approach would work with actual lobule morphology (e.g. Drasdo et al.)

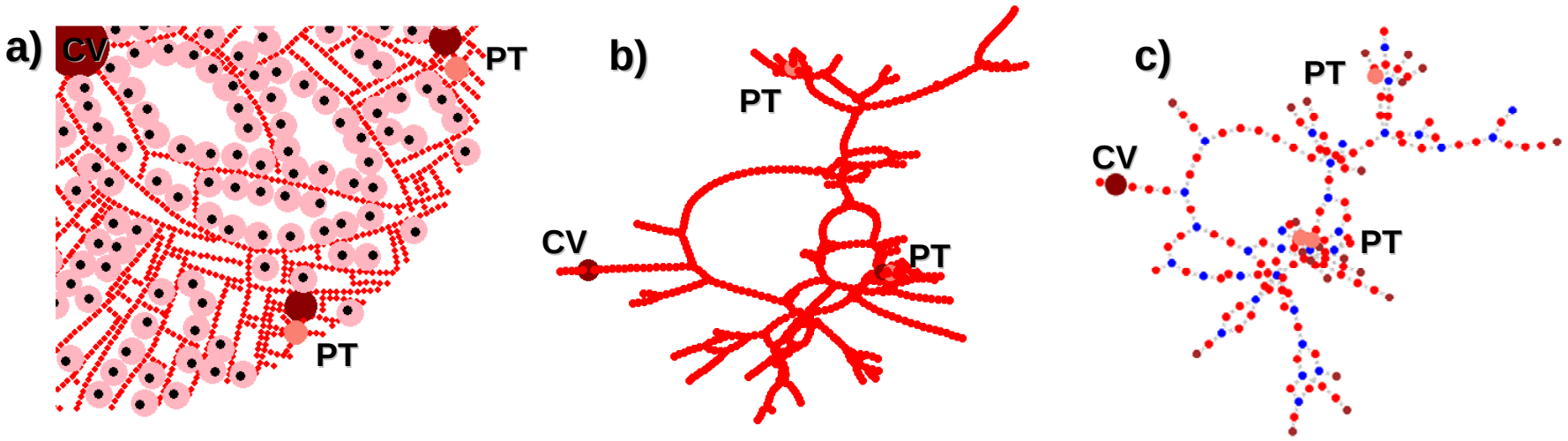
photo credits: Rockett et al. (2006) Reproductive Toxicology ***22*** *647-658*

Integration with Pharmacokinetic Models



- Our lobule module is directly integrated with a pharmacokinetic model.
- Determines specific amount of chemical delivered to any given hepatocyte in μM.
- Suitable for endogenous and xenobiotic compounds.

A Model for Tissue Microdosimetry

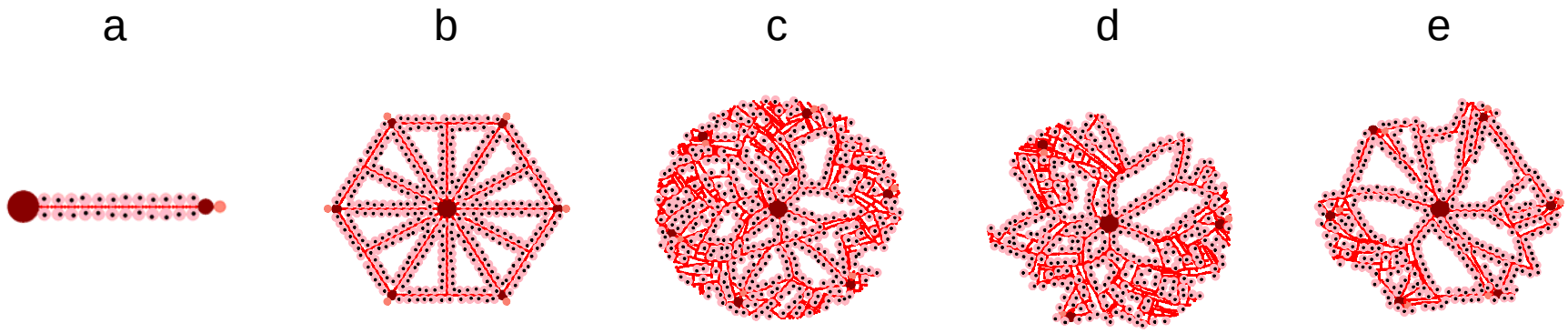


- A graph describes the connectivity between small, approximately well-mixed regions of sinusoids

- Flow (which determines rate constants) is described by simplified hemodynamics (Barnes (1980) Archives of Surgery **115**, 216-223)

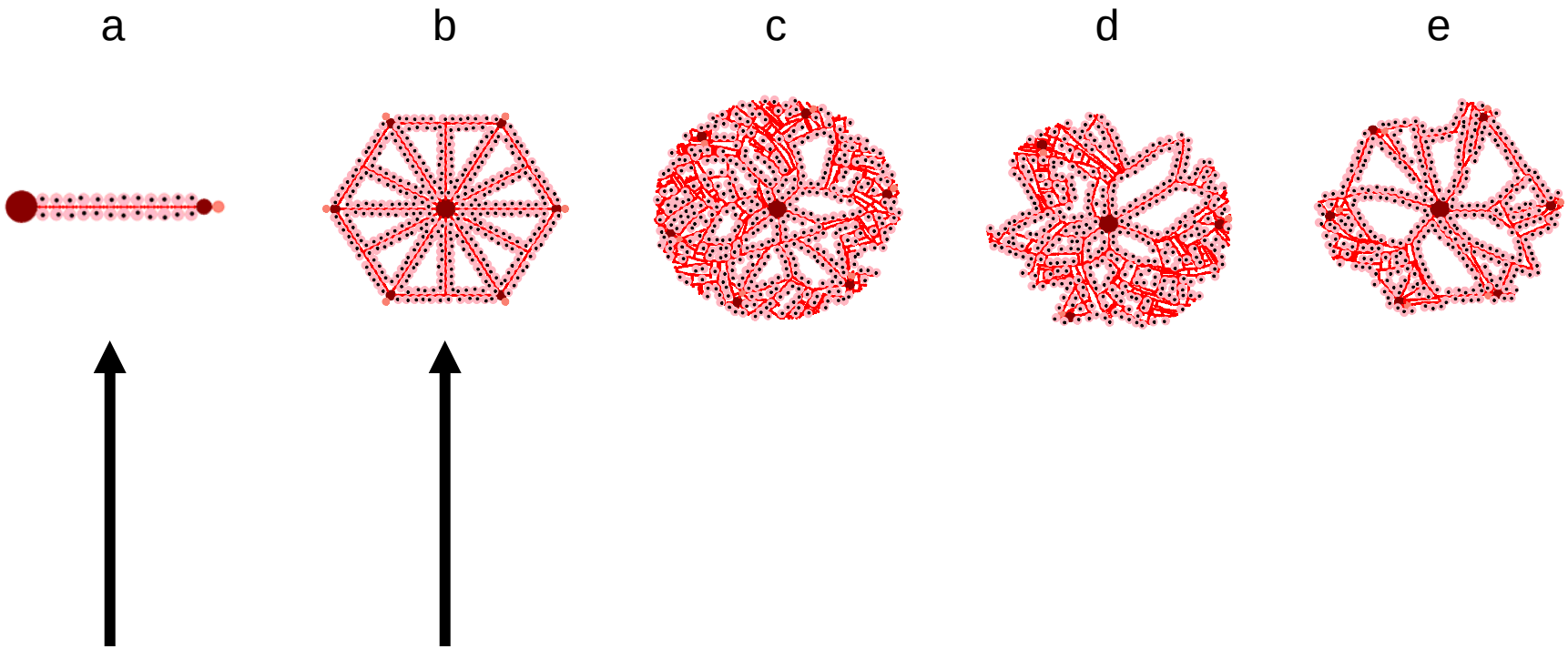
- A system of first-order equations gives the concentration for each sinusoid

Lobule Geometry



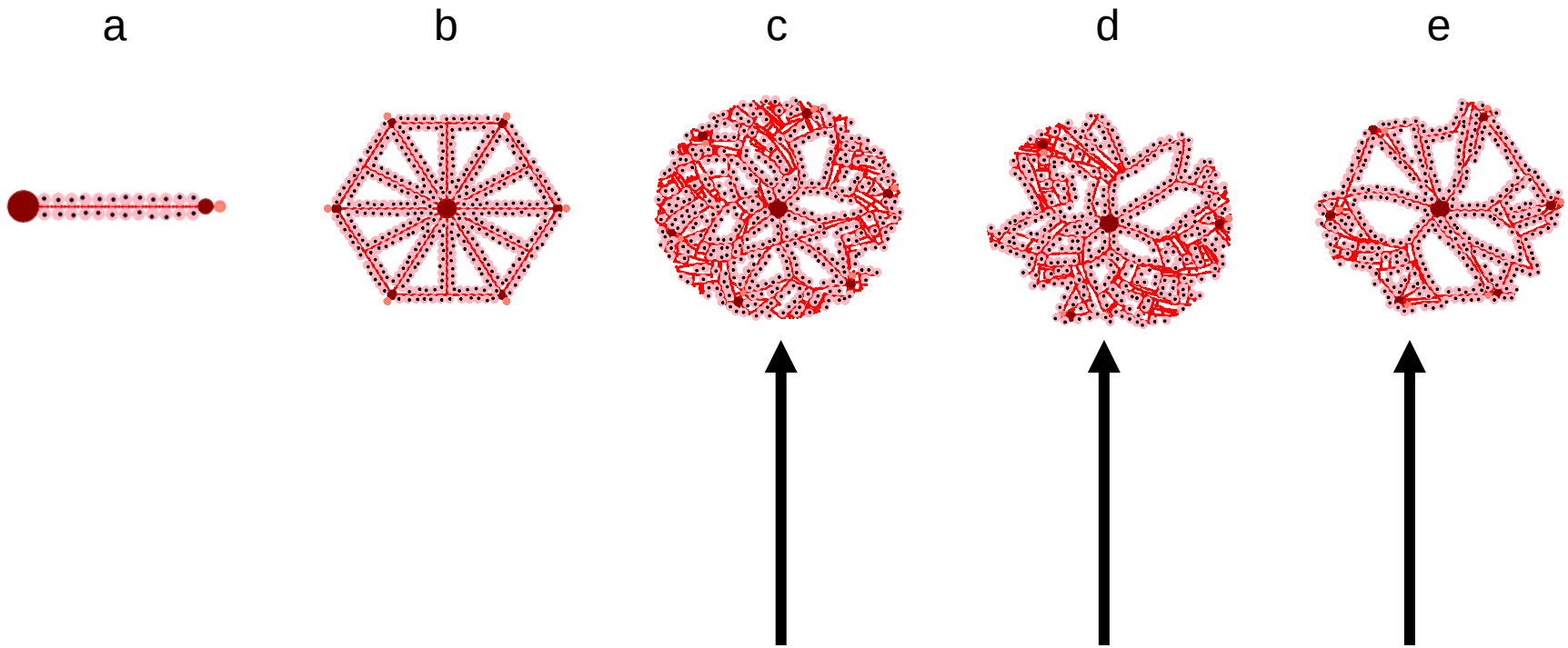
- Approach should work for a wide range of geometries
- We have studied a variety of geometries, awaiting quantitative data on morphometry of sinusoids (e.g., Drasdo et al.)
- Two dimensional assumption changes connectivity
- We studied variability in sinusoid branching and direction
- Other important variability exists (Crawford et al. (1998) Hepatology **28**, 323-331)

Lobule Geometry



Without random noise we approach the parallel tubes model

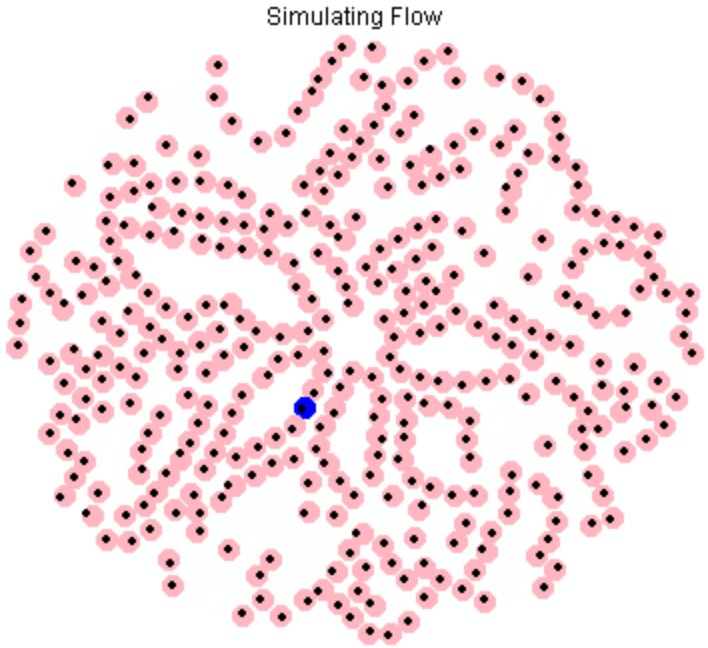
Lobule Geometry

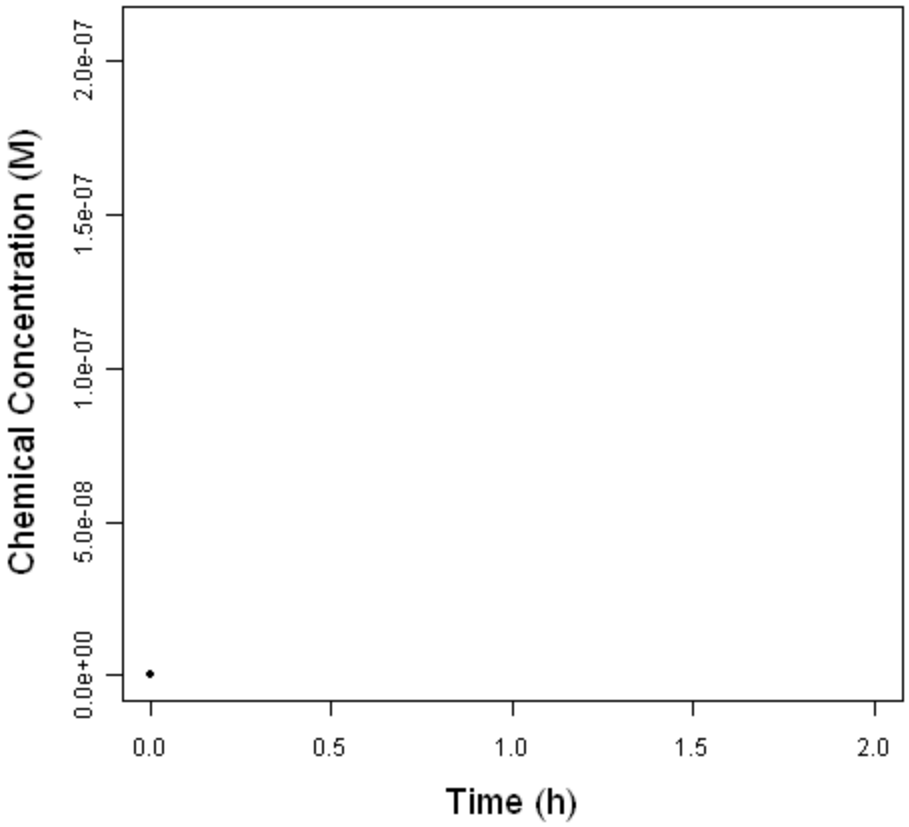


For more “physiologic” appearances we introduced **placement variability** and varied the number of portal triads and branching rate

Example of Compound Distribution

Predictions for single oral dose of 10 μMol
(0.03 mg/kg for 200 MW substance and 70 kg subject; Cl_{int} = 1 μL/min/10^6 hep.)





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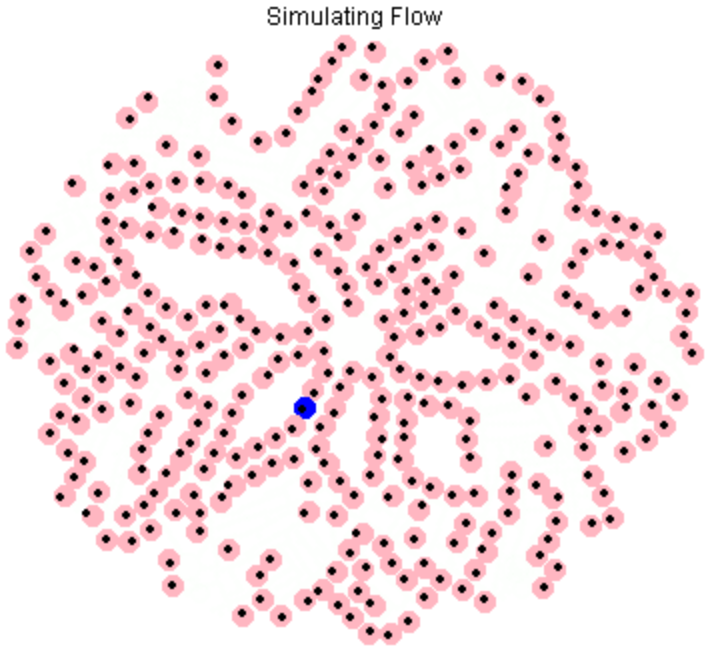
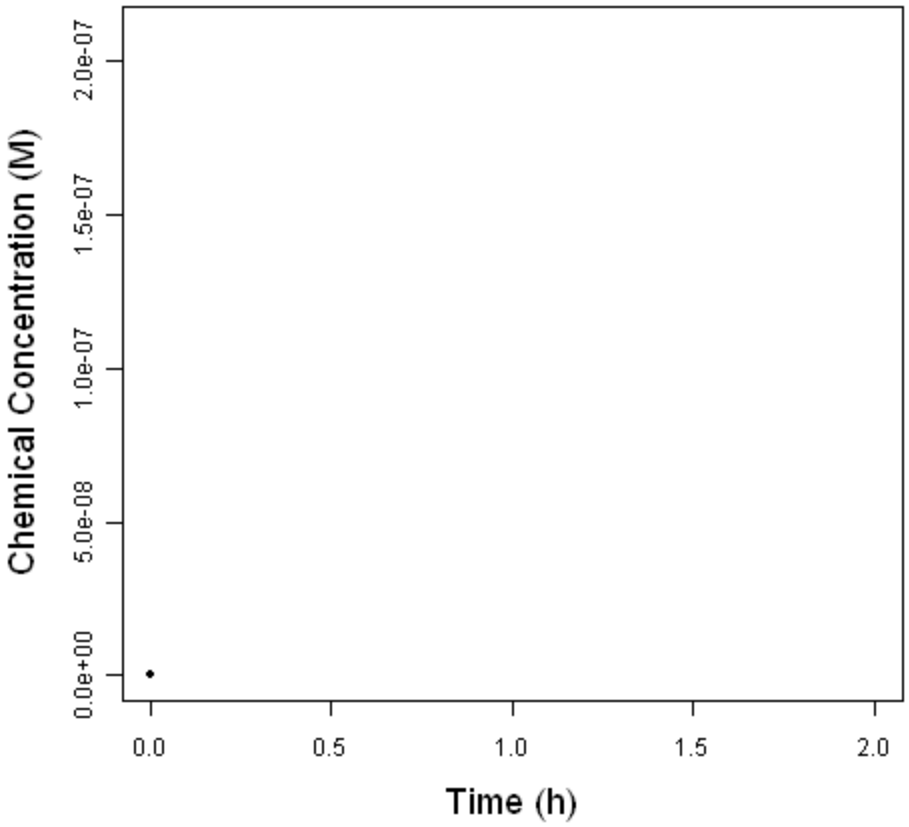
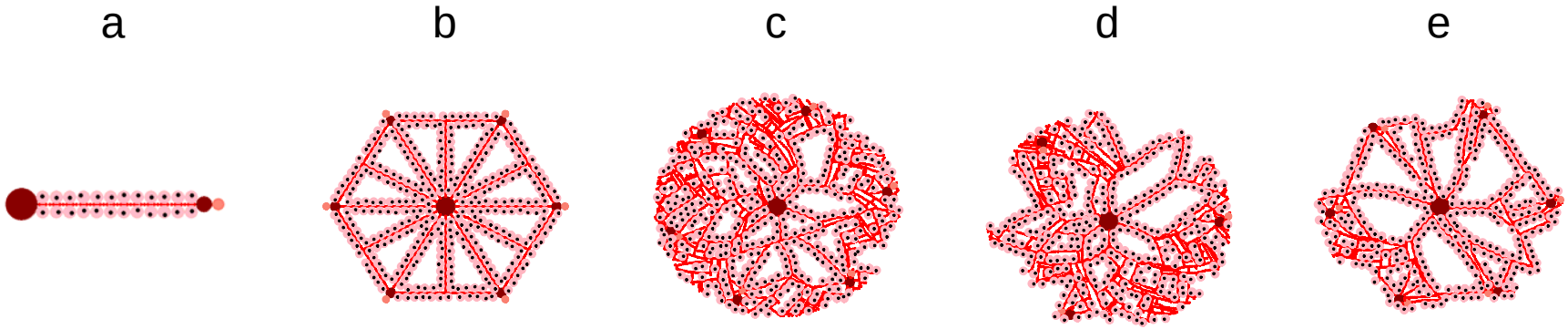


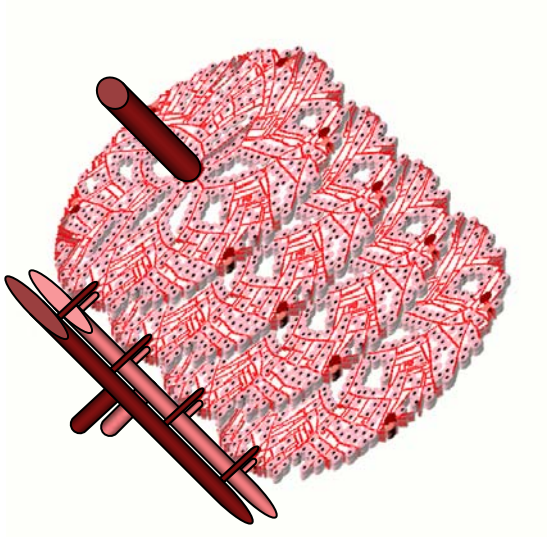


Millions of Parallel Lobules



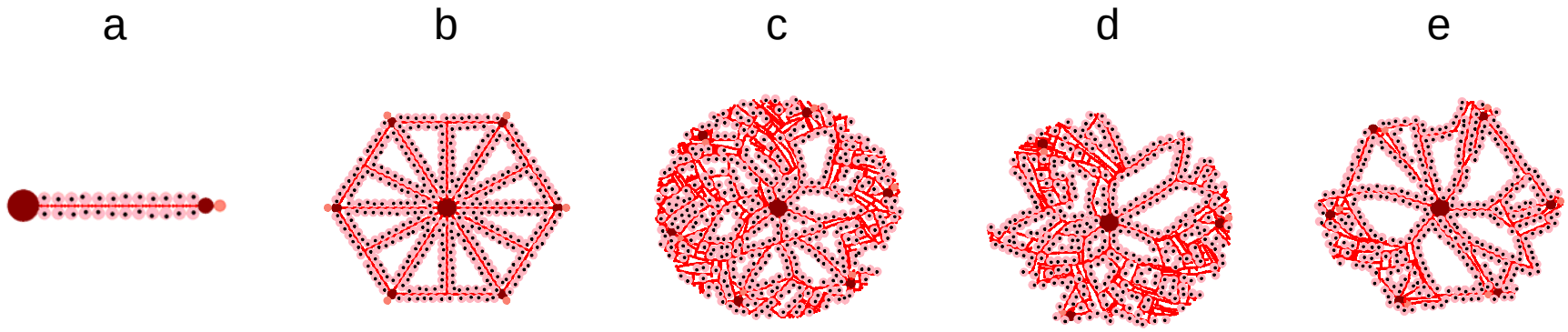
Human liver consists of roughly one million lobules (~1 mm^3 each)

Each simulated lobule has thickness of single sinusoid (~25 μm)



To preserve mass balance, millions of replicate “lobules” are needed to handle flow into the liver

Millions of Parallel Lobules



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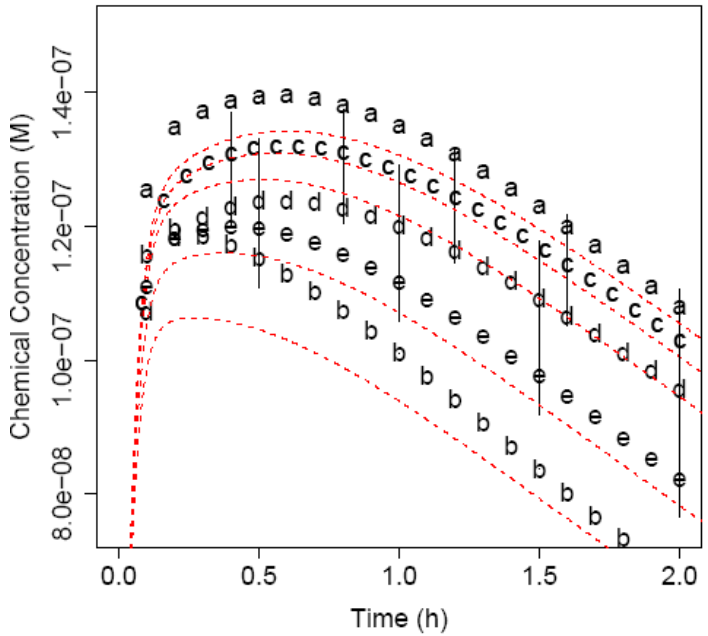
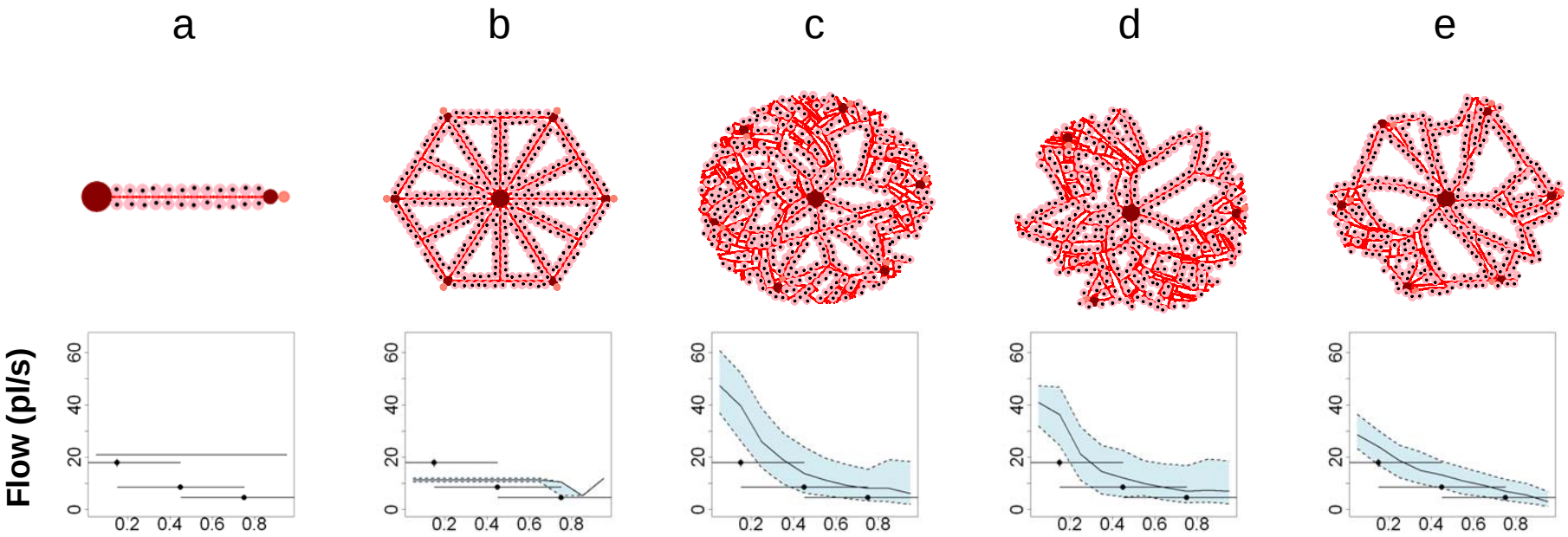


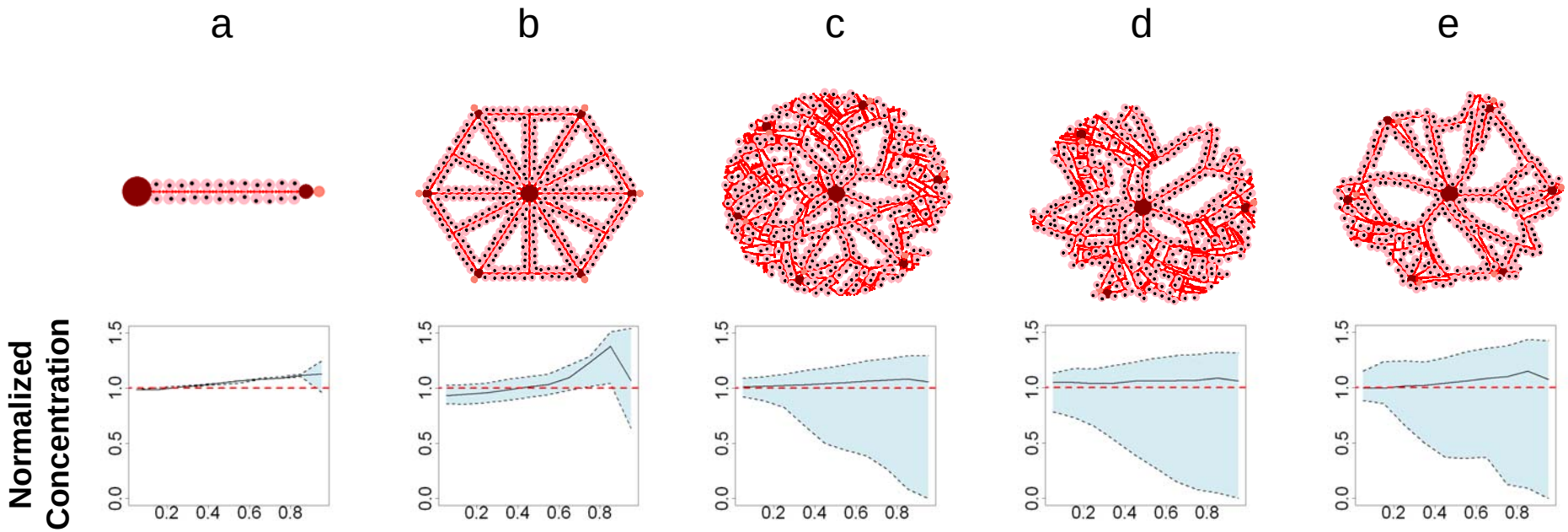
Dashed line indicates prediction for well-mixed PBPK compartment with equivalent metabolic clearance.

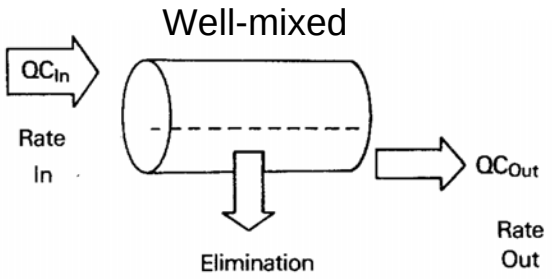
Variability Across the Lobule



Observations of flow from Komatsu et al. (1990) Microvascular Research **40**, 1-13

Variability Across the Lobule





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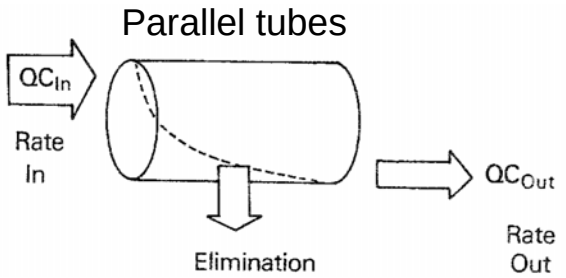
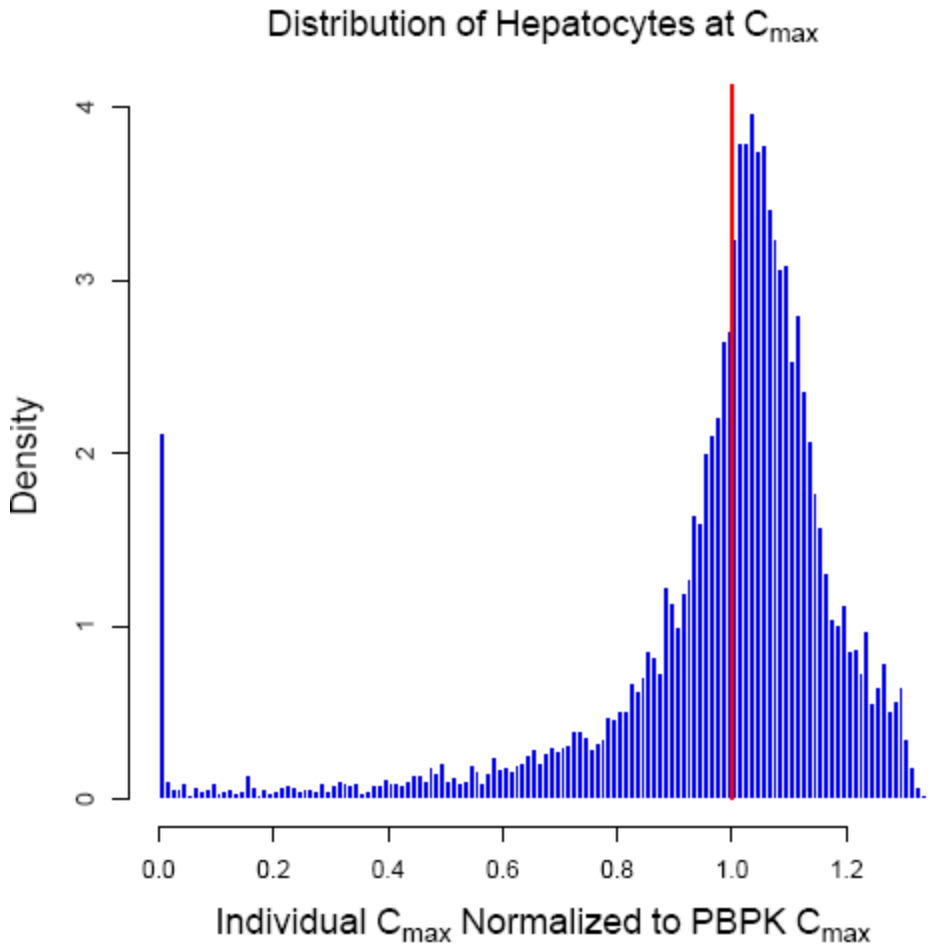
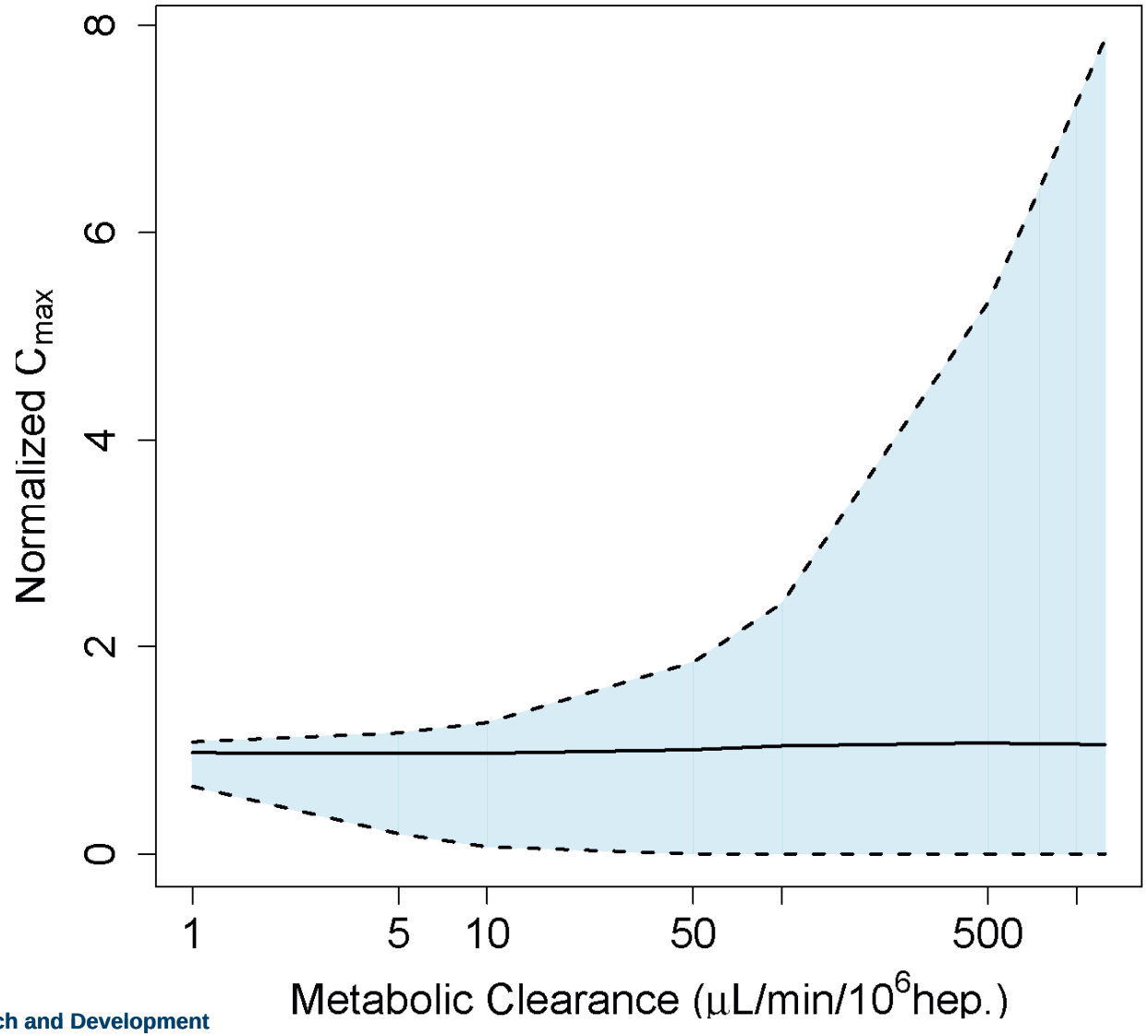


Variability on a Hepatocellular Level



Variability Depends on Rate of Metabolism



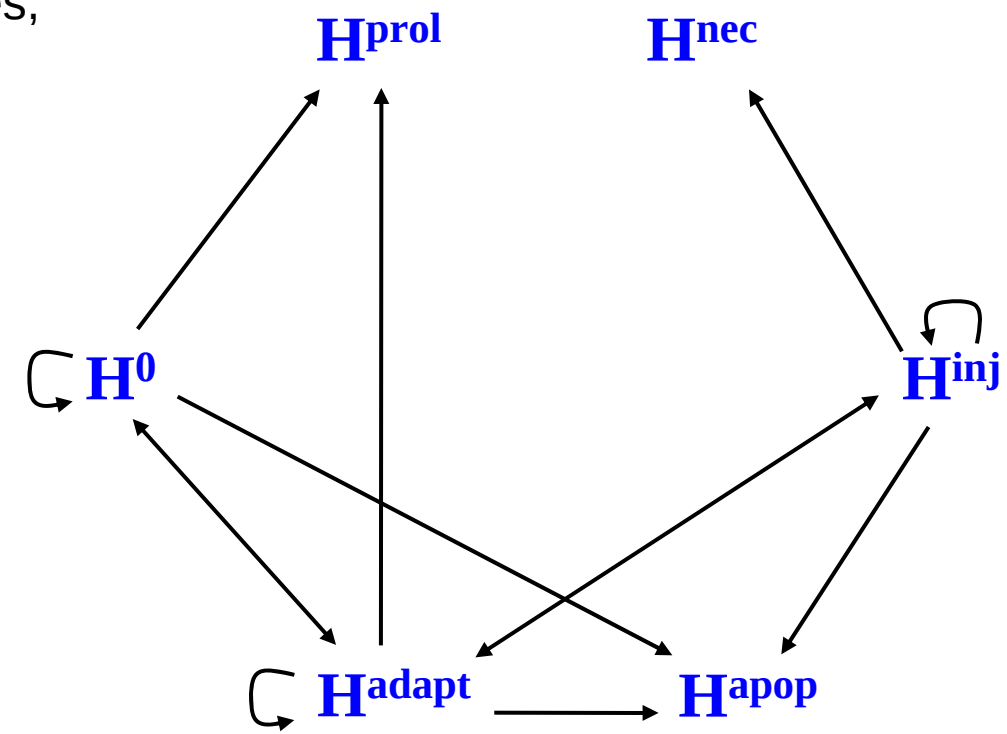
Consequences of Spatial Heterogeneity

Preliminary, placeholder network of cell state changes is being used in the tissue simulator

Six terminal and intermediate states, connected by transition rates

Transition rates can change in the presence of a compound

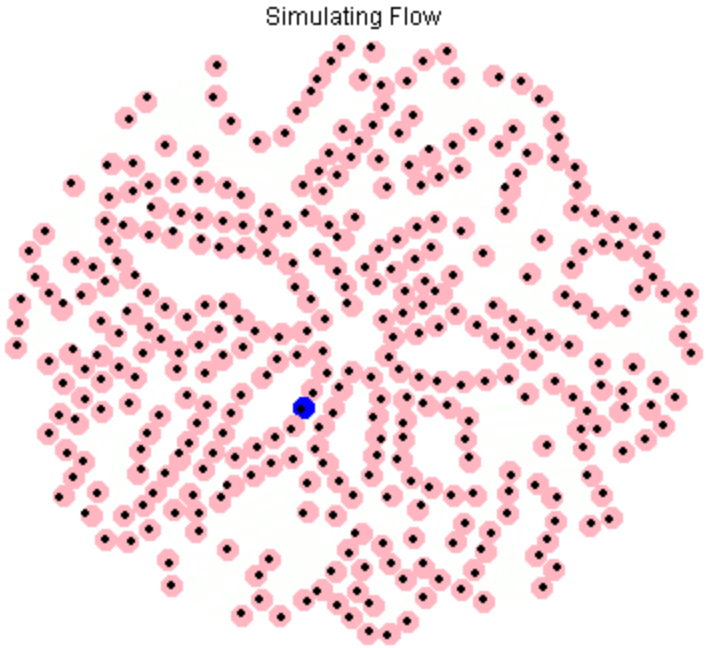
- H^0 (Normal/Quiescent)
- H^{adapt} (Stressed/Adaptive)
- H^{inj} (Stressed/Injured)
- H^{nec} (Necrotic)
- H^{prol} (Proliferative)
- H^{apop} (Apoptotic)



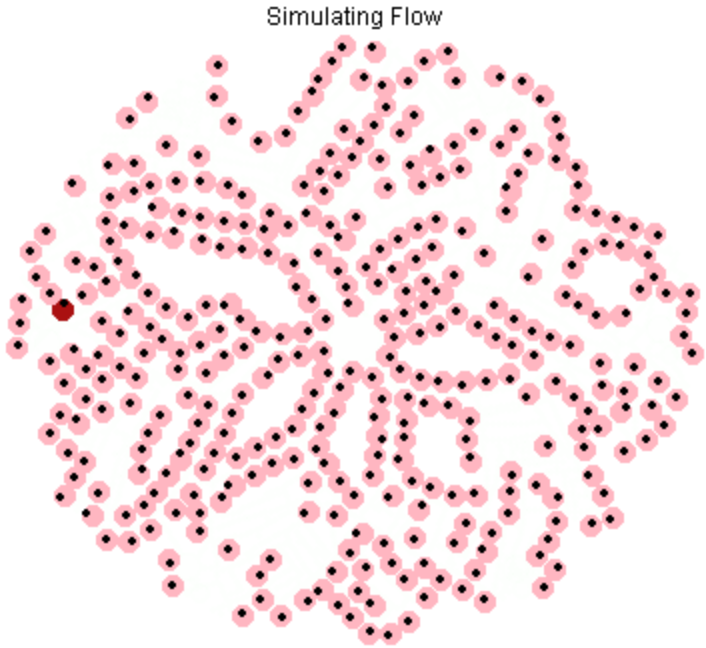
Cytotoxicty Threshold 110% PBPK C_{max}

United States Environmental Protection Agency

Slow Metabolism
(Cl_{int} = 1 μL/min/10^6 hep.)



Rapid Metabolism
(Cl_{int} = 1000 μL/min/10^6 hep.)



Concentration
Above Threshold
Below Cytotoxicity Threshold

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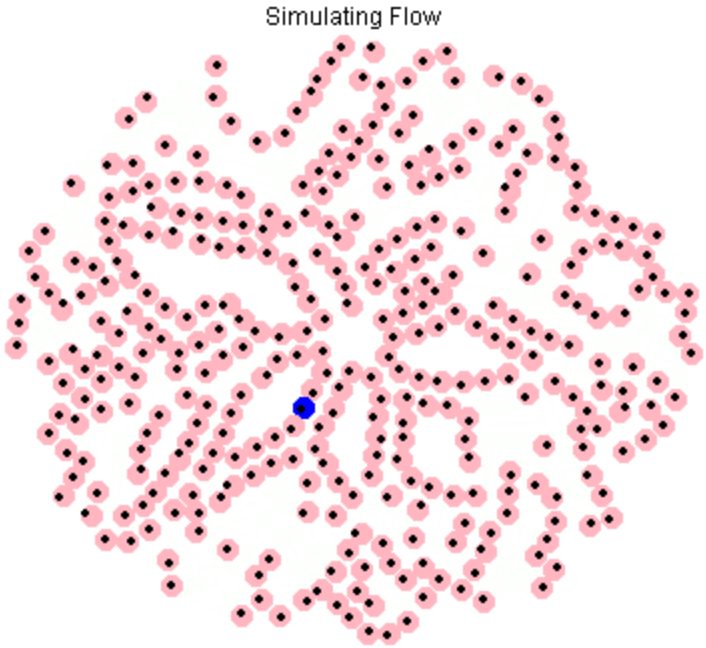

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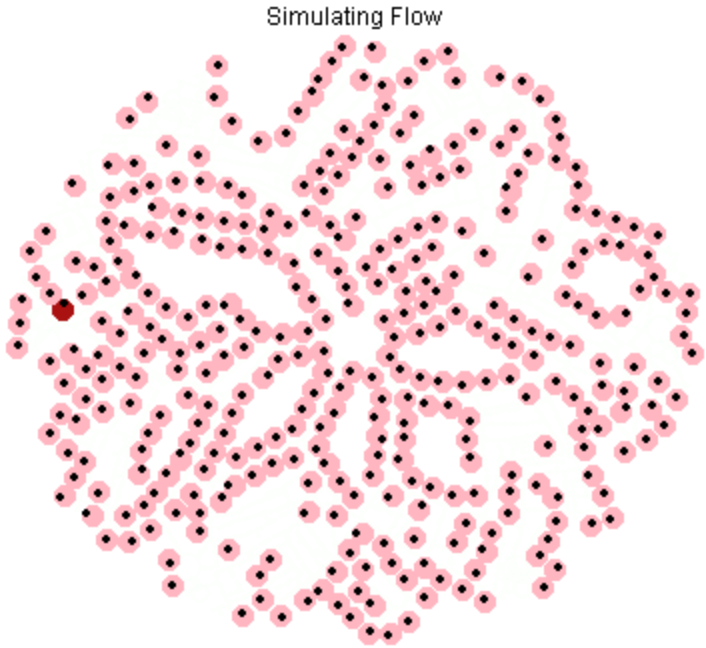

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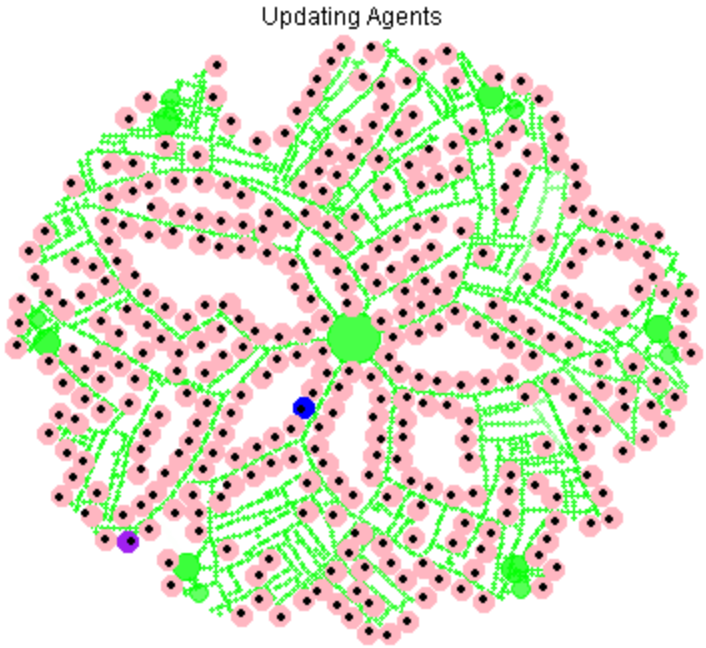

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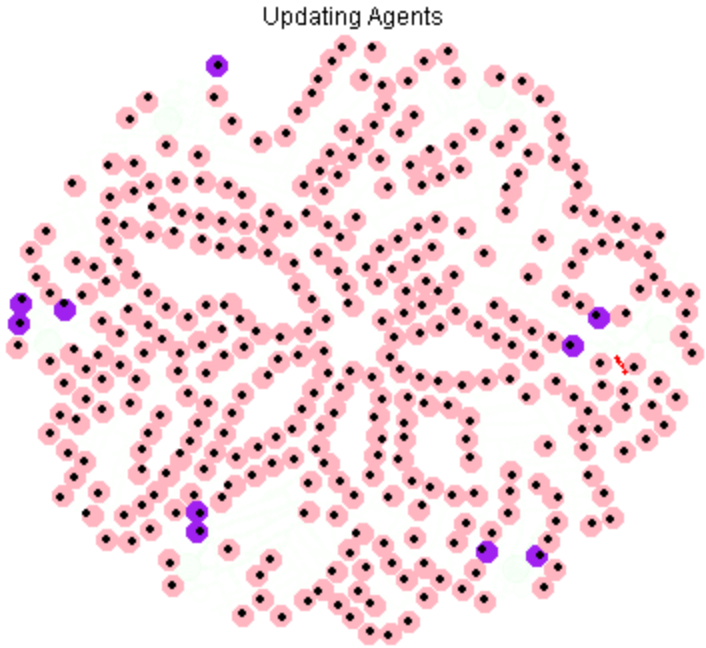

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Future Directions

- Track endogenous compounds such as oxygen, nutrients, hormones
- Demonstrate homeostasis by simulating emergent zonation of metabolism
- Develop dynamic model of cellular homeostasis that is perturbable by nuclear receptor activation
- Multiple chemical study of compounds with known hepatoxocity as well as environmentally-relevant compounds with available *in vitro* and pharmacokinetic data

National Center for Computational Toxicology
Virtual Tissues Researchers

Virtual Liver
Imran Shah
John Jack
Chris Haugh
Woody Setzer

KnowledgeBase
Amar Singh

Virtual Embryo
Tom Knudsen
Nicole Kleinstreuer
Nisha Sipes
Michael Rountree
Rob Dewoskin

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