# Biomimetic in Silico Devices

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**Abstract.** We introduce biomimetic in silico devices, and means for validation along with methods for testing and refining them. The devices are *constructed* from adaptable software components designed to map logically to biological components at multiple levels of resolution. In this report we focus on the liver; the goal is to validate components that mimic features of the lobule (the hepatic primary functional unit) and dynamic aspects of liver behavior, structure, and function. An assembly of lobule-mimetic devices represents an in silico liver. We validate against outflow profiles for sucrose administered as a bolus to isolated, perfused rat livers. Acceptable in silico profiles are experimentally indistinguishable from those of the *in situ* referent. This new technology is intended to provide powerful new tools for challenging our understanding of how biological functional units function *in vivo*.

## 1 Introduction

Cells of the same type in the same tissue can experience different environments, and as a consequence exhibit quite different gene expression patterns [1]. This is just one of the problems faced when modeling biological systems at multiple levels of resolution. Network detail learned from experiments on isolated cells *in vitro* may not map directly to the tissue level. Reflecting on this reality, Noble rejects the reductionist bottom-up and the traditional top-down modeling and simulation approaches [2]. He makes the case for a "middle-out" strategy that focuses on the "functional level between genes and higher level function" [3], and calls for new ideas and new approaches to help move the field to the next level. The new class of biological analogue models presented here, which we refer to as biomimetic in silico devices (hereafter, devices), is an answer to that call. The devices are designed to generate biomimetic behaviors and are constructed from software components that map logically to biological components at multiple levels of resolution. The focus

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here is the liver. The data used for validation are outflow profiles from experiments on isolated, perfused rat livers (hereafter, perfused livers) given bolus doses of compounds of interest [4, 5]. Acceptable devices are hepato-mimetic in that they generate in silico outflow profiles that are experimentally indistinguishable from those of the *in situ* referent.

## 2 Device Design

### 2.1 Modeling Approach and Biological Data

Rather than the traditional inductive, analytic modeling approach, we used a constructive approach based in part on ideas and concepts from several sources, including compositional modeling [6]. We focus more on the aspects of structure and behavior that give rise to the data. We deconstruct the system into biologically recognizable components and processes that can be represented as software objects, agents, messages, and events. Next, we reconstruct using those objects within a software medium that handles probabilistic events, and can represent dynamic spatial heterogeneity. The process produces in silico *devices* [6] capable of biomimetic behaviors. Of course, these devices are also models. We use *device* to stress their modular, constructive nature, to emphasize the essential properties discussed below, and to distinguish them from traditional equational models.

The devices represent aspects of the anatomic structure and behavior of the functional unit of the liver that influences administered compounds. We conduct in silico experiments that follow protocols that mimic the original *in situ* experimental protocols. Because a device is not based on equations, we do not directly fit it to data. We use a Similarity Measure [7] to quantify the similarity between data generated by the device and data generated by the biological referent. Having completed that level of validation we run simulation experiments to address what-if questions and/or *grow* the device so that it accounts for additional, different data (e.g., perfused liver outflow profiles of additional compounds and/or hepatic imaging data).

### 2.2 Properties: Essential and Desired

We create a device from data with as few assumptions as possible, by first building and validating a simple, biomimetic device, and then iteratively improving it. Devices and their components must be reusable, revisable, and easily updatable when critical new data becomes available, without having to re-engineer the whole device. Device components, like their biological referents, need to be sufficiently flexible and adaptable to be useful in a variety of research contexts. They should be able to function at multiple levels of resolution, from molecule to organ. In addition, device and components must logically map to their biological counterparts. Spatial heterogeneity is a quintessential characteristic of organisms at each organizational level. So, it is essential that device components be capable of representing that heterogeneity at different levels of resolution as required by the problem. Finally, hepatic processes, drug disposition, and pharmacokinetic processes are characterized

by probabilistic events. So, our biomimetic devices are exclusively event driven and most events can be probabilistic. These properties are deemed essential in part because they are expected to make this new technology easily accessible and useful to a majority of biomedical researchers.

### 2.3 Histological and Physiological Considerations

Changes in the architecture of hepatic fluid flow are associated with several disease states, and such alterations can influence a drug's disposition. These considerations suggest that a device must have a flexible means of representing that architecture at whatever level of detail is needed. We use directed graphs, with objects placed at graph nodes, to represent that architecture. Because the lobule is the primary structural and functional unit of the rat liver [8], the device must have a component that maps directly to the lobule. Hereafter refer to that component as a LOBULE[1].

Hepatocytes exhibit location-specific properties within lobules, including location-dependent expression of drug metabolizing enzymes [1]. Such intralobular heterogeneity requires that a LOBULE be capable of easily exhibiting heterogeneity and zonation when such properties are required. LOBULES must also be capable of representing specialized cell types, including endothelial, Kuppfer, and stellate cells, and their specialized behaviors in appropriate relative relationships, when needed, without forcing restructuring or redesign. The blood supply for one lobule, illustrated schematically by the cross section in Fig. 1, feeds into several dozen sinusoids that merge as they feed into the lobule's central vein (CV). Known hepatic features that are not needed to account for outflow profiles of the targeted data sets do not have corresponding components within the LOBULES. Examples include a separate hepatic arterial blood supply, the biliary system, and drug transport systems (into cells and into bile).

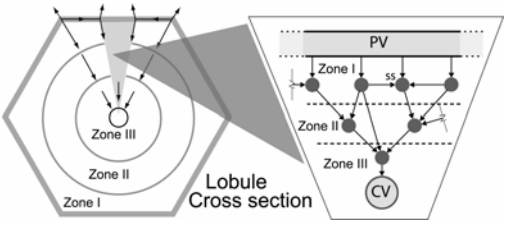


**Fig. 1.** A schematic of an idealized cross-section of a hepatic lobule showing half an acinus and the direction of flow between the terminal portal vein tract (PV), and the central hepatic vein (CV). SS: Sinusoidal Segment

### 2.4 Designing the in Silico Components

**Computational Framework.** Our devices are constructed within the Swarm framework (www.swarm.org). The methods do not require any particular formalism. But, the experimental framework is always formulated using Partially Ordered Sets; they are a generic way to specify concurrent processes with as few strictures as possible [9].

[1] When referring to the in silico counterpart of a biological component or process, such as "lobule," "endothelial cell," or "partition," we use SMALL CAPS.

**Directed Graphs.** A trace of flow paths within one lobule sketches a network that we represent by an interconnected, directed graph. Literature data [8] are used to constrain the accessible graph structures used. We only consider the subset of graphs that has more nodes connected to the portal vein tract (PV) (source) and fewer nodes connected to the CV (exit). Teutsch et al. [8] subdivide the lobule interior into concentric zones. For now, we impose a three-zone structure and require that each zone contain at least one node and that a shortest path from PV to CV will pass through at least one node and no more than one node per zone. The insert in Fig. 1 illustrates a portion of a graph that connects in silico PV outlets to the CV. Graph structure is specified by the number of nodes in each zone and the number of edges connecting those nodes. Edges are further identified as forming either inter-zone or intra-zone connections. The CV receives solute from the last node in each shortest path between PV and CV. Edges specify "flow paths" having zero length and containing no objects. A solute object exiting a parent node is randomly assigned to one of the available outgoing graph edges and appears immediately as input for the downstream child node. Randomly assigned intra-zone connections are allowed but are confined to Zones I and II. We randomly assign nodes to each of the zones so that the number of nodes in each zone is approximately proportional to the fraction of the total lobule volume found in that zone.

**Sinusoidal Segments and the Fate of Solutes.** Agents called sinusoidal segments (SSs) (Fig. 2) are placed at each graph node. There is one PV entrance (effectively covering the exterior of the LOBULE) and one CV exit for each LOBULE. A solute object is a passive representation of a chemical as it moves through the in silico environment. The PV creates solute objects, as dictated by the experimental dosage function, and distributes them to the SSs in Zone I. A solute object moving through the LOBULE represents molecules moving through the sinusoids of a lobule, and their behavior is dictated by rules specifying the relationships between solute location, proximity to other objects and agents, and the solute's physicochemical properties. Each solute has dose parameters and a scale parameter (molecules per solute object). The relative tendency of a solute object to move forward within a SS determines the effective flow pressure and this is governed by a parameter called *Turbo*. If there is no flow pressure (*Turbo* = 0), then solute movement is specified by a simple random walk. Increasing *Turbo* biases the random walk in the direction of the CV.

We have studied the behaviors of several sinusoidal segment designs and describe here the extensible design currently in use. Simpler designs generate behaviors that fail to meet our Similarity Measure criterion. Viewed from the center of perfusate flow out in Fig. 2, a SS is modeled as a tube with a rim surrounded by other layers. The tube and rim represent the sinusoidal space and its immediate borders. The tube

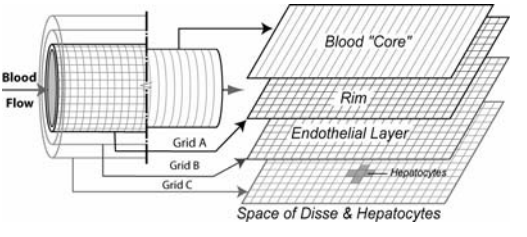


**Fig. 2.** Schematic of a sinusoidal segment (SS). Three types of SS are discussed in the text. One SS is placed at each node of the directed graph within each LOBULE

contains a fine-grained abstract Core space that represents blood flow. Grid A is the Rim. Grid B is wrapped around Grid A and represents the endothelial layer. Another fine-grained space (Grid C) is wrapped around Grid B to collectively represent the Space of Disse, hepatocytes, and bile canaliculi[2]. If needed, hepatocytes and connected features such as bile canaliculi can be moved to a fourth grid wrapped around Grid C. The properties of locations within each grid can be homogeneous or heterogeneous depending on the specific requirements and the experimental data. Objects can be assigned to one or more grid points. For example, a subset of Grid B points can represent one or more Kupffer cells. Objects that move from a location on a particular grid are subject to one or more lists of rules that are called into play at the next step. Within Grid B a parameter controls the size and prevalence of FENESTRATIONS[1] and currently 10% of Grid B in each SS is randomly assigned to FENESTRAE; the remaining 90% represents cells. Similarly, within the grid where some locations map to hepatocytes, there is a parameter that controls their relative density.

**Classes of Sinusoidal Segments and Dynamics Within.** To further enable accounting for sinusoidal heterogeneity, we defined two classes of SSs, $S_A$ and $S_B$. Additional classes can be specified and used when needed. Relative to $S_B$ the $S_A$ have a shorter path length and a smaller surface-to-volume ratio, whereas the $S_B$ have a longer path length and a larger surface-to-volume ratio. The circumference of each SS is specified by a random draw from a bounded uniform distribution. To reflect the observed relative range of real sinusoid path lengths, SS length is given by a random draw from a gamma distribution having a mean and variance specified by the three gamma function parameters, $\alpha$, $\beta$, and $\gamma$.

Solute objects can enter a SS at either the Core or the Rim. At each step thereafter until it is METABOLIZED or collected it has several stochastic options, the aggregate properties of which are arrived at through Monte Carlo simulation. In the Rim or Core it can move within that space, jump from one space to the other, or exit the SS. From a Rim location it can also jump to Grid B or back to the Core. Within Grid B it can move within the space, jump back to Grid A or to Grid C. When it encounters an ENDOTHELIAL CELL within Grid B it may (depending on its properties) PARTITION into it. Once inside, it can move about, exit, bind or not. Within Grid C it can move within the space or jump back to Grid B. When a HEPATOCYTE is encountered the SOLUTE can (depending on its properties) PARTITION into it or move on. Once inside a HEPATOCYTE it can move about, exit, bind (and possibly get METABOLIZED) or not. Currently all objects within a HEPATOCYTE that bind can also METABOLIZE. The probability of a solute object being METABOLIZED depends on the object's properties. Once METABOLIZED the object is destroyed. The only other way to exit a SS is from the Core, Rim or into bile (not implemented here). When the SOLUTE exits a SS and enters the CV, its arrival is recorded (corresponding to being collected), and it is destroyed.

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[2] Because we are building a normalized model there is no direct coupling between grid points within the fine-grained space and real measures such as hepatocyte volume or its dimensions in microns.

### 2.5 Similarity Measure

The *in situ* liver perfusion protocol is detailed in [4]. Briefly, a compound of interest is injected into the entering perfusate of the isolated liver. The entire outflow is collected at intervals and the fraction of the dose within is determined. The $^{14}$C-sucrose outflow profile contains information only about features of the extracellular environments whereas the data for a drug contains information on those features as well as on intracellular environments in that same liver. When the results of in silico and *in situ* experiments are similar, an expert can inspect the two data sets and offer an opinion on the degree of their similarity. However, automated model generation and refinement requires having one or more Similarity Measure (SM) to substitute for the expert's judgment. A SM is a function which takes two sets of experimental data and returns a number as a measure of their similarity [7]. Classical regression approaches do not apply because we are comparing the outputs of two or more experiments.

There are two main contributors to intraindividual variability: methodological and biological. For replicate experiments in the same liver the coefficient of variation for fraction of dose within specific outflow collection intervals typically ranges between 10 and 40%. A coefficient of variation can define a continuous interval bounding the experimental data. Any new set of results that falls within those bounds and has essentially the same shape is defined as being experimentally indistinguishable. The same should hold even if the data comes from an in silico experiment, and that provides the basis for selecting and evaluating SMs.

The objective of the SM is to help select among device designs, not simply to specify a device and select among variations on that device. Hence, the successful SM must target the various features of the outflow profile that correlate with the generative structures and building blocks inside the device. However, for simplicity in these early studies we have assumed that the coefficients of variation of repeat observations within different regions of the curve are the same. In that way we can use a simple interval SM, which is what we do. A set of *in situ* outflow profiles, $T$, is used as training data. From this data, we calculate a distance, $D$, from a reference that will be the basis for a match. We then take two outflow profiles and pick one to be the reference profile, $P^r$. For each observation in $P^r$, create a lower, $P^l$, and an upper, $P^u$, bound by multiplying that observation by $(1 - D)$ and $(1 + D)$, respectively. The two curves $P^l$ and $P^u$ are the lower and upper bounds of a band around $P^r$. The two outflow profiles are deemed similar if the second profile, $P$, stays within the band. The distance $D$ used for sucrose is one standard deviation of the array of relative differences between each repeat observation and the mean observations at that time. To calculate $D$ for the training set $T$ we choose experimental data on different subjects that were part of the same protocol [4, 5].

## 3 In Silico Experimental Results

To begin development of a new device we select an outflow profile and begin the process of finding the simplest design, given restrictions: one is that it be comprised

of the minimum components needed to generate an acceptably similar profile. An initial unrefined parameterization is chosen based on available information. Components are added according to that initial parameterization. If the behavior of the resulting device is not satisfactory, any given piece of the device may be surplused and replaced, modified, or reparameterized with minimal impact on the other components within the device. This process continues until the device provides reasonable coverage of the targeted solution space. Once an acceptable parameterization is found, the parameter space is searched further [10] for additional solution sets. Bounds for the parameter space can be specified to indicate solution set regions for which the device validates (i.e., acceptable SM measures are obtained). Subsequently, for a second data set, a repeat of the first experiment or a data set for a second drug, we make only minimal adjustments and additions to the structure of the first device so that the resulting new device generates acceptable outflow profiles for both data sets.

The typical *in situ* outflow profile is an account of on the order of $10^{15}$ drug molecules percolating through several thousand lobules. The typical in silico dose for *one* run with one LOBULE is on the order of 5,000 drug objects, where each drug object can represent a number (• 1) of drug molecules. Thus, a resulting single outflow profile will be very noisy and will be inadequate to represent the referent *in situ* profile. Another independent run with that same device, parameter settings, and dose will produce a similar but uniquely different outflow profile. Changing the random number generator seed alters the specifics for all stochastic parameters (e.g., placement of SSs on the digraph), thus providing a unique, individual version of the LOBULE, analogous to the unique differences between two lobules in the same liver. A full in silico experiment is one that produces an outflow profile that is sufficiently smooth to use the SM, and typically combines the results from 20 or more independent runs using the same LOBULE.

One can identify several similar parameterizations that will yield outflow profiles that are experimentally indistinguishable from each other. That is because there is a region of device-structure space (model space) that will yield acceptable behaviors relative to a specific data set. Such a region can be viewed as a metaphor for the fact that all lobules are similar but not identical. We currently do not attempt to fully map acceptable regions of either model or parameter space. Our goal is simply to locate a region in each that meets our objectives.

Figure 3 shows results from one parameterization of a LOBULE against a perfused liver sucrose outflow profile. The shaded region is a band enclosing the mean fraction of dose collected for each collection interval. The width of the band is ± 1 std about

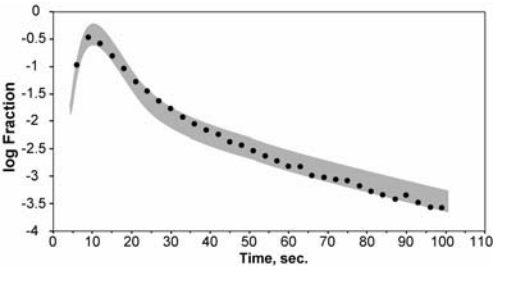


**Fig. 3.** An outflow profile for a device parameterized to match a sucrose outflow profile. Nodes per Zone: 55, 24 and 3 for Zones I, II and III, respectively; total edges: 60; intra-zone connections: Zone I = 10, Zone II = 8, Zone III = 0; inter-zone connections: I → II = 14, I → III = 4, II → III = 14; SSs: 50% $S_A$ and $S_B$; Number of runs = 100

the mean. The filled circles are results obtained using the specified device parameter vector.

## 4 Higher and Lower Levels of Resolution

Different regions of a normal liver are indistinguishable. That is generally not the case for a diseased liver, where some lobules can be damaged or otherwise changed. So, to understand and account for such differences we need methods to shift levels of resolution without loss of information. The following summarizes how we are enabling resolution changes. To represent a whole diseased liver with heterogeneous properties we can first connect in parallel four to five different sized lobes, where each is a directed graph having multiple parallel, single node paths connecting portal and hepatic veins nodes (Fig. 4), and agents representing secondary units are placed at those nodes. A lobe is comprised of a large number of these units [8]. Each secondary unit can be similarly represented by a directed subgraph with LOBULES placed at each of its nodes (insert, Fig. 4). When subcellular networks within CELLS located in Grids B and C are needed, they may also be treated as directed graphs with nodes representing factors and with edges representing interactions and influences [11]. Having a mechanism for realizing networks allows us to replace sub-networks (at any location) with rules-based software modules.

## 5 Conclusion

We have tested and affirmed the hypothesis that perfused liver outflow data obtained following bolus administration of sucrose can, in conjunction with other data, be used to specify and parameterize a physiologically recognizable hepato-mimetic device that can generate outflow profiles that are experimentally indistinguishable from the original *in situ* data. Each device is *constructed* from software components that exhibit several essential properties including being designed to map logically to hepatic components at multiple levels of resolution, from subcellular to whole organ. This new technology is intended to provide powerful tools for optimizing the designs of real experiments. It will also help us challenge our understanding of how mammalian systems function in normal and diseased states, and when stressed or confronted with interventions.

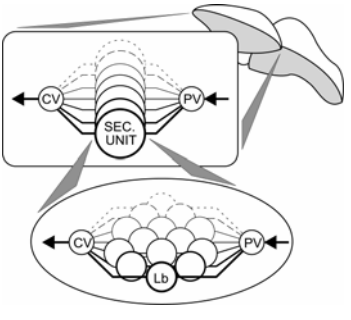


**Fig. 4.** An illustration of the hierarchical structure of an in silico liver. A lobe is comprised of a network of secondary units (SEC.UNIT) [8]; they, in turn are comprised of a network of lobules (Lb) as pictured in Fig. 2. PV: Portal vein. CV: Central hepatic vein

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