A Liver-centric Multiscale Modeling Framework for Xenobiotics

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Why develop computational models?

• Codify (and test) biological knowledge
• Identify gaps in knowledge
• Reduce the use of experimental animals
• Enable *in vitro* to *in vivo* extrapolation (IVIVE)
Why Model the Liver?

• Key organ in many important human diseases (obesity, hypercholesterolemia, type II diabetes, hepatitis, ...)

• Major site of drug toxicity (drug induce liver toxicity DILI)

• Major site of drug metabolism and clearance
Why Model Acetaminophen?

• Acetaminophen (APAP, aka Paracetamol) is a widely used over the counter pain reliever and fever reducer.
• Is a leading cause of liver failure in the USA.
• A typical orally active pharmacological agent with large amounts of both human and animal data.
• The therapeutic index (ratio of toxic to therapeutic doses) is unusually small for an over the counter medication.
• Is included in many formulations and often a patient isn’t aware that the use of APAP, in conjunction with a prescribed pain killer (like Percocet), leads to a high and perhaps lethal APAP doses.
• Is often used as the typical compound for Drug Induced Liver Injury (DILI) studies.

\[
\text{N-acetyl-para-aminophenol}
\]
APAP Metabolites in Humans

**Phase I**
- APAP → NAPQI
  - CYP mediated N-hydroxylation and rearrangement

**Phase II**
- NAPQI → NAPQI-GSH
  - GSH Conjugation
- APAP → APAP-Sulfate
  - Sulfation
- APAP → APAP-Glucuronide
  - Glucuronidation

GSH 3-10mM
**APAP Metabolites in Humans**

**Phase I**
- CYP mediated N-hydroxylation and rearrangement
- GSH Conjugation

**Phase II**
- Sulfation
- Glucuronidation

Major pathways at therapeutic dose.
APAP Metabolites in Humans

Phase I

CYP mediated N-hydroxylation and rearrangement

APAP

NAPQI

GSH

3-10mM

(or cellular proteins)

Phase II

Sulfation

APAP-Sulfate

Glucuronidation

APAP-Glucuronide

Toxicity pathway at high dose.
APAP Metabolites in Humans at therapeutic dose

Given data such as this, can the tissue and cell level concentrations be calculated?

Multiple scales, ranging from the entire body down to individual chemical reactions, contribute to the effects of a chemical. Computational tools exist to model the behavior of chemicals at each of these biological scales. Integration of the individual tools creates a multiscale model that can represent the multifaceted nature of chemical effects. To apply the multiscale model to a new chemical entity, or to a new pathway, requires effective tools for mining the large quantity of publicly available data.
So Models need to be Multiscale

Length Scale

Whole body (and population) Organ Tissue Cell Subcell

Biology (in vivo)

Computation (in silico)

Reactions:

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Source ODE:</th>
</tr>
</thead>
<tbody>
<tr>
<td>A → NA;</td>
<td>$V_{max_A} A/(K_m_A + A)$</td>
</tr>
<tr>
<td>NA+GSH → NAGSH;</td>
<td>$k_{NaGsh} NA \times GSH$</td>
</tr>
<tr>
<td>$X_1$ → GSH;</td>
<td>$k_{Gsh} (GSH_{max} - GSH)$</td>
</tr>
<tr>
<td>A → Ac;</td>
<td>$V_{max_{II_A}} A/(K_m_{II_A} + A)$</td>
</tr>
</tbody>
</table>

12/5/2017
Modeling Workflow

1. Biological Experiments
2. Biological Observations
3. BioModel
4. Mathematical Model
5. Computational Model
6. Simulation
7. Prediction

Verification

Validation

New Knowledge
Developing a multiscale, multimodal model
Multiscale models –
Models at individual scales should be:

– Built using standard tools and specifications for that scale
– Annotated
– Shareable and reusable
– Falsifiable
– “Runnable” on their own
Advantages of using existing tools and standards

• Can use existing tools for model—
  – creation
  – execution
  – parameter fitting
  – *etc.*

• Can re-use existing models

• Can use tools that include annotation standards
A multiscale, liver-centric model
Multiscale model of APAP Pharmacokinetics

Physiologically based pharmacokinetic modelling (PBPK)

Virtual Tissue (VT)

Subcellular reaction kinetics (RK) or signaling network

12/5/2017
Whole Body
PBPK Model
ADME: Adsorption-Distribution-Metabolism-Excretion

Typical data available for drug exposure in an experimental animal or human

- How much knowledge can be extracted from this experimental data?
- Given just this blood concentration versus time data predict tissue concentration and biological response
Whole-Body PBPK Model fit to ADME Data
Systems Biology Markup Language (SBML)

- Describes biological processes that can be modeled as a set of ordinary differential equations (ODEs).
- Shareable and annotatable.
- Large number of tools to build and simulate SBML models.

SBML.org
SBML is annotatable
SBML is annotatable

Basic PBPK (Physiologically Based Pharmacokinetic) model of Acetaminophen.

This is a basic model of Acetaminophen (APAP, Paracetamol) pharmacokinetics in humans. Many of the model parameters (compartment volumes, volumetric flow rates, etc.) are scaled allometrically based on the body weight (BW) raised to the 3/4 power. Because of that, the assigned values of many of the parameters are recalculated at run time and are different than the default values for the particular entity (e.g., the volume of a compartment and the volumetric flow rate between compartments).

APAP dose is initially given in grams (APAP_Dose_gran), which is converted to moles via the APAP molecular weight (APAP_MW). APAP quantities throughout the rest of the models are given in moles.

The base parameters are for a 70Kg human and a pharmacological oral dose of 1.4 gram of APAP. Metabolism is modelled as a single ODE in the liver compartment and the metabolite does not leave that compartment.
Lobular organ which has as its parts lobules connected to the biliary tree.
SBML is shareable
Whole-Body PBPK Model fit to ADME Data

**Biological Model (right):** Compartment model for oral administration of APAP in humans (pharmacological dose).

**Mathematical Model (below):** PBPK ODEs in Jarnac syntax.

<table>
<thead>
<tr>
<th>Transfer</th>
<th>Source ODE</th>
</tr>
</thead>
<tbody>
<tr>
<td>CArterial</td>
<td>QArterial*CArterial/VArterial;</td>
</tr>
<tr>
<td>CArterial</td>
<td>QLiver*CArterial/VArterial;</td>
</tr>
<tr>
<td>CArterial</td>
<td>QKidney*CArterial/VArterial;</td>
</tr>
<tr>
<td>CArterial</td>
<td>QRest*CArterial/VArterial;</td>
</tr>
<tr>
<td>CLung</td>
<td>QCardiac*CLung/VLung;</td>
</tr>
<tr>
<td>AGutlumen</td>
<td>kGutabs*AGutlumen;</td>
</tr>
<tr>
<td>CVen</td>
<td>QCardiac*CVen/VVen;</td>
</tr>
<tr>
<td>CRest</td>
<td>QRest<em>CRest</em>Ratioblood2plasma/KRest2plasma/Fraction_unbound_plasma/VRest;</td>
</tr>
<tr>
<td>CGut</td>
<td>QGut*CGut/VGut;</td>
</tr>
<tr>
<td>CLiver</td>
<td>(QLiver+QGut)<em>CLiver</em>Ratioblood2plasma/Kliver2plasma/Fraction_unbound_plasma/VLiver;</td>
</tr>
<tr>
<td>CKidney</td>
<td>Qgfr*CKidney/Kkidney2plasma/VKidney;</td>
</tr>
<tr>
<td>CKidney</td>
<td>QKidney<em>CKidney</em>Ratioblood2plasma/Kkidney2plasma/Fraction_unbound_plasma/VKidney;</td>
</tr>
<tr>
<td>CLiver</td>
<td>CLmetabolism*CLiver/Kliver2plasma/Fraction_unbound_plasma/VLiver;</td>
</tr>
</tbody>
</table>
PBPK Parameter Summary

Species, age, gender, ... specific parameters

Compound specific parameters

**Variables:** These are amounts (grams)
- CArt
- CGut
- CKidney
- CLiver
- CMetabolized
- CLung
- CRest
- CTubules
- CVen

**Parameters:** Compartment volumes (L)
- VArt
- VGut
- VKidney
- VLiver
- VLung
- VRest (rest of body)
- VVen

**Parameters:** Rates and partition coeffic.
- Qgfr
- Fraction_unbound_plasma (Fub or Fup)
- kGutabs
- Kkidney2plasma
- Kliver2plasma
- KRest2plasma
- CLmetabolism
- Ratiodblood2plasma (Rb2p)
Whole-Body PBPK Model in SBML/COPASI

https://www.ebi.ac.uk/biomodels-main/BIOMD0000000619
Whole-Body PBPK Model in COPASI: Parameter Fitting
Whole-Body PBPK Model in COPASI: Parameter fitting results

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Lower Bound</th>
<th>Start Value</th>
<th>Value</th>
<th>Upper Bound</th>
<th>Std. Deviation</th>
<th>Coeff. of Variation [%]</th>
<th>Gradient</th>
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<tr>
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<td>0.01</td>
<td>1</td>
<td>3.75238</td>
<td>1000</td>
<td>354.108</td>
<td>9436.89</td>
<td>-2.57628e-13</td>
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<td>Values[kGutabs].InitialValue</td>
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<td>100</td>
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<tr>
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<td>1</td>
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<tr>
<td>Values[QGFR_ref].InitialValue</td>
<td>1e-06</td>
<td>1</td>
<td>0.741127</td>
<td>1</td>
<td>44.7509</td>
<td>6038.22</td>
<td>4.23772e-12</td>
</tr>
</tbody>
</table>
Whole-Body PBPK Model fit to ADME Data

Standalone Run (right): Simulation results (solid line) for the PBPK model in SBML for a 1.4g oral APAP dose in a 70 Kg human. Open symbols are average data for a group of human patients with average body weight of 70 Kg. [1]

Liver
(organ / tissue)
model
Liver Topology:
The liver is a massively parallel device with complex microvasculature.
Divide and Conquer Build

1a

1b

Dissociation of Drug via Metabolism

Arterial

Lung

Rest

Kidney

Gut

Tubules

CLmetab

Metab.

Venous

Liver

Q_{Arterial}

Q_{Rest}

Q_{Kidney}

Q_{GFR}

Q_{Gut}

Q_{Liver}

Q_{Liver}+Q_{Gut}

CompuCell3D

3a

3b

Phase II

Phase I

APAP

APAP-Sulfate

APAP-Glucuronide

Phase I

NAPQI

GSH

NAPQI-GSH

CYP-mediated N-dealkylation and rearrangement
Cellular Potts Model

- Developed by James Glazier and Francois Graner
- Represents single cell as a collection of pixels on a lattice (regular or irregular) in 2D or 3D.
- **Physics based.**
- Uses **energy formalism** to describe cell properties (usually in the form of constraints), cell-cell interactions and cells’ behaviors.
- **Stochastic** not, deterministic
- Very simple and intuitive. Mathematical complexity does not go beyond understanding second order polynomials.
- **Capable of representing cellular and subcellular structures**
- Relatively fast up to $10^5$ cells on a single CPU.
- Used by many labs around the world.
- **Drawbacks:** Time has to be converted from simulation unit to physical unit. Lattice granularity determines level of details of simulated objects. Simulation parameters are not independent—changing one of them triggers changes in others
- **Software:** **CompuCell3D**, Chaste, Simmune, Morpheus, Tissue Simulation Toolkit

\[ E = \sum_{\text{neighbors}} J \left( \tau(\sigma(x)), \tau(\sigma'(x)) \right) \left( 1 - \delta(\sigma(x), \sigma'(x)) \right) \]

\[ + \sum_{\sigma} \lambda_s(\sigma) \left( s(\sigma) - S_{\text{Target}}(\sigma) \right)^2 \]

\[ + \sum_{\sigma} \lambda_i(\sigma) \left( v(\sigma) - V_{\text{Target}}(\sigma) \right)^2 \]

\[ + E_{\text{chem}} + E_{\text{hapt}} + ... \]

\[ P(\Delta E) = 1, \: \Delta E \leq 0 \]

\[ P(\Delta E) = e^{-\Delta E/kT}, \: \Delta E > 0 \]

[www.compucell3d.org](http://www.compucell3d.org)
CompuCell3D can incorporate subcellular reaction modelling

- Biochemical Kinetics:
  - Cell-Cycle
  - Circadian rhythms
  - Cardiac rhythms
  - cAMP oscillations
  - Delta-Notch patterning
  - WNT pathway
  - FGF pathway
  - Etc...
Tissue (multicell) scale model

Better than a simple PBPK compartment but not as complex as real liver vasculature
Multicell Sinusoid Model

A single liver sinusoid is modeled in CompuCell3D (CC3D). The CC3D model describes the behavior of the cells and blood in the model. Blood is modeled as a mixture of serum portions and red blood cells (RBCs).

**Biological Model:** Hepatocytes (top & bottom), Red Blood Cells (RBCs, dark green), Serum Portions (blue), Blood "source" cells (red). Blood is forced into the model from the left and exits on the right. RBCs and Serum Portions carry a chemical "load" of APAP that diffuses into / out of the serum, RBCs and Hepatocytes as shown at the right. Transfer requires, and is scaled by, contact.
Standalone Run: Shown are three time points of the CC3D model of a square input pulse of APAP into the model sinusoid. Cells and serum portions are colored by the amount of APAP. The top image shows the pulse entering the portal (left) end of the sinusoid. The middle and bottom images show the peak pulse and the washout period after the end of the pulse.
Multicell Sinusoid Model
Subcellular metabolism (reaction kinetic) model
Subcellular Reaction Kinetics Model
**Subcellular RK Model**

**Biological Model (right):** Simplified Phase I and Phase II metabolism of APAP.

**Model (below):** Reaction kinetic (RK) ODEs in SBML/COPASI syntax.
Subcellular RK Model in COPASl

**Standalone Run:** Simulation results for the reaction kinetic model, for a single hepatocyte, in Jarnac. The vertical axis is mM and the time axis is seconds.

https://www.ebi.ac.uk/biomodels-main/BIOMD00000000624
Subcellular RK Model in COPASl

**Standalone Run:** Simulation results for the reaction kinetic model, for a single hepatocyte, in Jarnac. The vertical axis is mM and the time axis is seconds.

Possible to calibrate this scale based on *in vitro* data (IVIVE).

https://www.ebi.ac.uk/biomodels-main/BIOMD0000000624

This ratio is known from human ADME studies.
Complete Multiscale Model
Complete Multiscale Model

Replicates for:
1. APAP
2. APA-Glucuronide
3. APAP-Sulfate

Three diffusing species:
1. APAP
2. APA-Glucuronide
3. APAP-Sulfate

1. Replicate in each of the 20 hepatocytes
2. “Well stirred” containers
3. Converts APAP to the three metabolites
Complete Multiscale model

Comp cell 3D acts as the controlling program and loads, initializes, time steps and communicates with the SBML components.

In a simulation step, the CC3D script sequences both the calculations it carries out directly as well as time stepping the linked SBML models.

A computational time step cycle consists of:

1. Time step the PBPK models updating the quantities of APAP, APAPG and APAPS in each compartment of the PBPK module.
2. Fetch the quantities of APAP within "CArt" and "CGut" compartments of the PBPK model and calculate the concentration of APAP entering the inlet side of the tissue-level (CC3D) sinusoid module.
3. The CC3D model creates blood portions and RBCs representing venous and arterial flow that contain the amount of APAP (or APAPG or APAPS) based on the blood concentrations in the two blood flows in the whole-body PBPK models.
4. The CC3D model then simulates blood flow for a short period of time.
5. Numerically integrate the transfer of drug molecules at the interface of blood (serum portions and RBCs) and hepatocytes.
6. Time steps the subcellular SBML models within each hepatocyte, which generates new values for the per-cell concentrations of APAP and metabolites.
7. At the central vein terminus of the sinusoid the APAP (and metabolites) are returned to the "CVen" compartment exit of the PBPK module.
Complete Multiscale Model Results

Time: 00 Hours 00 Minutes

APAP Concentration (ug/mL)

Flow Direction

APAPG Concentration (ug/mL)

APAPS Concentration (ug/mL)
Parameter Estimation

• Initial set of parameters based on parameter fitting in the individual sub models and literature values.

• Parameters refined using a combination of random sampling about the initial parameter set followed by finer random sampling around the best candidate parameter sets.
Complete multiscale model of APAP Pharmacokinetics: Parameter estimation challenges

• 35 parameters
• 36 measurements in the human ADME data
• 3 output variables (serum concentrations vs time)
• Dozens of internal variables (e.g., APAP concentration in individual cells)

• Parameter interactions
• Parameter identifiability
• Uncertainty in the experimental data
Complete multiscale model of APAP Pharmacokinetics

The accuracy of the model can be judged based on how well it reproduces several different characteristics of the ADME curve ($t_{\text{max}}$, $C_{\text{max}}$, AUC) as well as by the RMS error between the entire simulated time course and the in vivo ADME data.
Complete multiscale model: Sensitivities for compound-specific parameters

Some sensitivities will optimally be zero at the solution!

Different model outputs have different sensitivities.
Complete multiscale model:
Compound-independent parameter sensitivities
Complete multiscale model:
Formation of NAPQI-GSH metabolite sensitivities

- APAP active transport into hepatocytes
- Phase II (conjugation)
- Phase I (oxidation)

PBPK | CC3D | Subcellular
Complete multiscale model:
Non-linear pairwise sensitivities

Some sensitivities will optimally be zero at the solution!
Complete multiscale model: Fixed Point Sensitivities

Sensitivities suggest which parameters need to be known with accuracy.
Some questions:

• Are the sensitivities measured in a particular sub-model the same as in the complete model?

• Do parameter fits for a particular sub-model help in fitting the complete model?

• Can parameter fitting, parameter exploration, sensitivity analysis etc. done at one scale help with the same processes at the complete multiscale?
Simulation of a Population

Serum APAP concentration for 1000 simulated individuals was generated by assuming that for each in silico individual, each parameter was within a truncated normal distribution with coefficient of variation of 25% around the base parameter set.

Comparison of the average response (closed symbols with error bars) of the simulated population with the simulated individuals that deviate the most, high and low, from the population average (symbols without error bars). Error bars are standard deviations.
Simulation of a Population

Reconstructed Critchley et al. with SEMs converted to SDs.

Human ADME data

Simulated population
Simulation of a Population

Reconstructed Critchley et al. figure with SEMs converted to SD.
Future
One from Menu A, one from Menu B, one (or more) from Menu C

**Menu A:**
- Whole Body
- Two-Compartment Model
- Multi-Compartment Model
- Multi-Compartment, Multi-Compound Model

**Menu B:**
- Organ
- Local 2-Compartment Model
- Simple Spatial Model
- Detailed 3D Model

**Menu C:**
- Cell
- Delta-Notch Signaling
- Xenobiotic Metabolism
- Homeostatic Metabolism
Future Work: A more realistic lobule model
Calculated flow velocities in a more realistic lobule model
Thank You

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- **Imaging Collaborators:** Dr. Kenneth Dunn
- **Toxicology Collaborators:** Dr. James Klaunig, Dr. Zemin Wang
- **Eye Collaborators:** Dr. Tom Gast
- **Kidney Collaborators:** Dr. Robert Bacallao

- **Support:** EPA, NIH, NSF, Indiana University.

For papers on these projects: http://www.biocomplexity.indiana.edu
To download software for model building: http://www.compucell3d.org
fini