

In Silico Pharmacokinetic System

The In Silico Pharmacokinetic System (ISPKS) is an agent-based, discrete-event, simulation library core designed to be the foundation for running in silico pharmacokinetic experiments. ISPKS is written in Java programming language, and supported by MASON (<http://cs.gmu.edu/~eclab/projects/mason/>), a fast, discrete-event multi-agent simulation library in Java. ISPKS provides the functionality to represent pharmacokinetic experiments computationally; executing the software simulates the experiments. The source code of ISPKS can be viewed at <https://subversion.assembla.com/svn/teddylam/>

The purpose here is to showcase the functionality and capability of the ISPKS, represented in two specific instantiations of the ISPKS. The two instances differ in their assembly of components, and hence they represent two different pharmacokinetic experiments.

- The first one is In Silico Drug Absorption Tract (“ISDAT”), representing human oral drug absorption in the context of a clinical bioavailability trial.
- The second is In Silico Enterocyte Monolayer model (“Interplay” model), used in studying enzyme-transporter interplay in an *in vitro* cell monolayer transport experiment.

Each instance is packaged into a Java Archive file (“JAR File”), which contains required Java and MASON libraries. Additional files required for post-simulation analyses are also included. Altogether these files are included in a compressed zip file on the attached CD. Demonstration videos are available for both instances. These files are also available at my website at

<http://www.pharmacy.cuhk.edu.hk/1/about-us/academic-staff/teddylam/?target=supplementary+information>

This document contains the installation and simulation guide for these two models, as well as recommended simulations to be carried out with each of them.

Installation Guide

1. **Download & install Java SE Development Kit (“Java SDK”) 8 or above.**

Java SDK is required to run the programs. The installer is available at <http://www.oracle.com/technetwork/java/javase/downloads/jdk8-downloads-2133151.html>

Please make sure to download and install the correct one for your Operation System and Platform

<u>Operation System / Platform</u>	<u>Required Java SDK file</u>
Mac OS X	jdk-8u101-macosx-x64.dmg
Windows x86 (32 bit)	jdk-8u101-windows-i586.exe
Windows x64 (64 bit)	jdk-8u101-windows-x64.exe

2. Java 3D (for Windows only):

Java 3D is required to run the 3D visualization from the graphical user interface (“GUI”). More information about Java 3D can be found here: <https://java3d.java.net/>. Java 3D installer is included with the zip files, and it is also available at <https://java3d.java.net/binary-builds.html>.

For 64 bit Windows systems, please install both

j3d-1_5_2-windows-i586.exe , and

j3d-1_5_2-windows-amd64.exe

whereas 32 bit Windows only needs to install the i586.

Java3D is not necessary for Mac OS X because we used the native Mac graphics libraries.

3. Download & install R and its related packages (for automatic analysis by R scripts)

R is a free software environment for statistical computing and graphics. It is required to run the automatic analysis scripts for ISDAT. More information about R can be found here: <https://www.r-project.org/>.

- a. Installers for R are available at <https://cran.r-project.org/>. Follow the link to download the precompiled binary distribution for your operating system
- b. Install the R software with the installer
- c. After R has been successfully installed, open the R console
- d. type in the following commands to install the required packages:
 - `install.packages("ggplot2")`
 - `install.packages("plyr")`
 - `install.packages("PK")`
- e. **For Windows:** Add R to the “PATH” environment variable

Go to control panel > Search for Environment Variable > Add the directory of *RScript.exe* to the variable *PATH*. (e.g. If the file of *RScript.exe* is located at “C:\Program Files\R\R-3.3.1\bin\x64”, then add this directory to the variable *PATH*)

4. ISPKS Files:

Extract the whole zip file to a destination directory (for example, extracting the zip file to the desktop), or just copy and paste the extracted folders (the ISDAT and Interplay folders), including all subdirectories, to the destination directory.

To start simulations using batch files

Windows users may double-click the batch files (.bat files) to start the simulations. Alternatively, follow the steps below.

To start simulation for ISDAT

	Windows	Mac OS
5.	Run the command prompt: Start > type “cmd.exe” > Enter	Open the terminal
6.	Go to the directory of the JAR file if the directory of isdat-win.jar is located at C:\User\SOP\Desktop\ISDAT, enter “cd C:\User\SOP\Desktop\ISDAT” in your command prompt	Go to the directory of the JAR file if the directory of isdat-macos.jar is located at /Users/SOP/Documents/workspace, enter “cd /Users/SOP/Documents/workspace”
7.	Execute the ISDAT JAR file type in “java -jar isdat-win.jar [custom parameters]” on command prompt	Execute the ISDAT JAR file type in “java -jar isdat-macos.jar [custom parameters]” on command prompt

<ul style="list-style-type: none"> - Custom parameters o -B1 = run midazolam with GUI o -B2 = run midazolam with auto analysis o -B3 = run clonazepam with GUI <p>Example: <i>"java -jar isdat-win.jar -B1"</i></p>	<ul style="list-style-type: none"> - Custom parameters o -B1 = run midazolam with GUI o -B2 = run midazolam with auto analysis o -B3 = run clonazepam with GUI <p>Example: <i>"java -jar isdat-macos.jar -B1"</i></p>
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NOTE: the programs may take about 30-60 seconds to start

To start simulation for Interplay

	Windows	Mac OS
5.	Run the command prompt: Start > type "cmd.exe" > Enter	Open the terminal
6.	Go to the directory of JAR file if the directory of interplay-win.jar is located at C:\User\SOP\Desktop\Interplay, enter <i>"cd C:\User\SOP\Desktop\Interplay"</i> in your command prompt	Go to the directory of the JAR file if the directory of interplay-macos.jar is located at /Users/SOP/Documents/workspace, enter <i>"cd /Users/SOP/Documents/workspace"</i>
7.	Execute the Interplay JAR file type in <i>"java -jar interplay-win.jar"</i>	Execute the Interplay JAR file type in <i>"java -jar interplay-macos.jar"</i>

NOTE: the programs may take about 30-60 seconds to start

- **If GUI is run (ISDAT -B1, -B3 and Interplay), [Requires Java3D]**
 - Click play to start the simulation.
 - The user may change model parameter values on "Model" tab of GUI Control before start of simulation. Please follow the number format when changing values.
 - The simulation may be paused and restarted at any time, using the "Pause" button.
 - To stop and reset the simulation, press the "Stop" button.
 - The graphical display can be rotated and zoomed in for better visualization. It is recommended to pause the simulation first before doing so.
 - Additional options about the display can be found under the "Console" tab
 - The user may inspect model components by clicking the objects on the display. Relevant information will be shown under the "Inspector" tab.
 - Raw simulated data can be found in csv files in subfolders in the "out" folder, after the simulation is stopped.
 - TIPS: for faster simulation, close the graphical display and the chart. To reactivate them, double click them under the "Displays" tab.

- **If auto analysis is run (ISDAT -B2), [Requires RScript.exe, and the ggplot2, plyr, and PK packages]**
 - The simulation will be repeated 10 times, and the results averaged
 - At the end of simulation, analysis scripts will be run to do mathematical operations
 - Graph and calculated result will be output to the "out/Report" folder. A comparison graph between simulated and reference data will be made, and AUC values, as well as statistical similarity values (RMSE) are calculated.
 - Raw simulated data can be found in csv files in subfolders in the "out" folder, after the simulation is stopped.

- NOTES: to start a new simulation and analysis, please remove the previous simulation results located inside “out/” and “out/Report” folders.

Recommended simulations for ISDAT

The ISDAT is created to simulate oral drug absorption, by simulating the mechanistic processes, ie, gastric emptying, intestinal motility, permeation into enterocyte, intestinal metabolism, first pass hepatic extraction, and so on. Because the ISDAT is very complex, in this simplified ISDAT simulation, only two sets of parameters are selected for manipulation, namely, the enzyme expression levels and the intestinal flow rate. In the “Model” tab, the user may change expression levels of enzymes in enterocytes and hepatocytes (duodenum, jejunum, ileum, and hepatocytes), and flow rate within the gastrointestinal lumen. The user may also select to simulate intravenous dose by checking the Ivdose check box.

The display shows the simulation of drug absorption. At the start of the simulation, drug objects (yellow dots) are placed in the stomach space; enzymes (purple cubes) in enterocytes and hepatocytes, and binders (blue cubes) in systemic blood. From the stomach space, drug objects move into the intestinal lumen and then move across the enterocytes and towards systemic blood, simulating oral absorption. Once the drugs are in the systemic blood, some are being bound to binders. Finally, during elimination, drugs objects are being converted into metabolite (white dots), predominantly in the hepatocytes.

The outcomes (measurements) of the simulation experiments are the profiles of parent drug and metabolite in systemic blood. The chart shows the amount of parent drug (red line) and metabolite (blue line) in the systemic blood space, and these can be mapped to observed concentration

- First, run the simulation, with GUI, with the default parameters for both midazolam (-B1) or clonazepam (-B3), note the approximate Tmax, Cmax and terminal slope.
- Repeat the simulation but slow down the flow rate parameters.
 - Since both midazolam and clonazepam are both highly permeable, decreasing the flow rate will slightly delay the Tmax
- Decrease the number of enzymes only the hepatocyte (The listed numbers are number of enzymes in duodenum, jejunum, ileum and hepatocytes, respectively)
 - Both drugs are extensively metabolized, the extraction by hepatocyte is the main route of elimination, therefore, the terminal slope would become much flatter, and Cmax will be higher. The effect on midazolam would be greater than that of clonazepam, because the latter is highly bound in systemic blood.
- Decrease the number of enzymes in both the enterocytes and hepatocytes
 - Cmax will be even higher, but little change on the terminal slope compared to the above
- Change the administration to intravenous dose (Check the IVdose box)
 - The profile will now mimic the exponential fall, and there is no Tmax or Cmax.
- Run the auto analysis scripts (-B2) and check the output graphs in the Report folder
 - Check the graphs to see the similarity between simulated and reference data for midazolam. The calculated PK parameters and statistical similarity values can be found in the Midazolam_params.csv file, which also show good similarity.

Recommended simulations for Interplay

The interplay model is created to study the enzyme-transporter interplay, and specifically to study how the interactions between passive permeability, affinity to enzymes and transporters, and expression levels of enzymes and transporter, affect a dual substrate's metabolic profile. The simulation mimics a transport study across a cell monolayer.

In the "Model" tab, 7 model parameters / experimental conditions can be changed:

Model parameters / experimental conditions	Representing...
InProb	Passive permeability of test compound
NumberCYP	Expression level of metabolizing enzymes
NumberPGP	Expression level of efflux transporters
CypAffinity	Affinity to enzyme of test compound
PgpAffinity	Affinity to transporter of test compound
B2ADose	Dosing direction: apical to basal or basal to apical
NumberCompound	Administered dose

The display shows the overview of the simulation. The top box is the apical space, followed by cellular space and basal space in the bottom. Efflux transporter (Pgp) is expressed on the apical membrane (green cones). Metabolizing enzymes (Cyp) are expressed inside the cellular space (purple cubes). Whenever a transporter or enzyme is occupied by a compound, its color turns into coral. If it is saturated, its color turns into orange. Parent compound is depicted as yellow dot and the metabolite is shown as white dot.

The outcomes (measurements) of the simulation experiments are the extraction ratio (ER), and fraction metabolized (Fmet). A dynamic time series chart shows both ER (blue line) and Fmet (red line), and a dynamic bubble chart shows the relationship between ER and Fmet. (The scale of the bubble should be set to a small number, like 0.0001)

- First, run the simulation with the default parameters, note the approximate ER and Fmet at around 360 steps.
- Repeat the simulations with different parameters, for example, increasing numberCYP (represents expression level of enzyme), or InProb (represents passive permeability). Again, note the ER and Fmet values at about 360 steps.
 - Increasing the number of enzymes will likely increase both ER and Fmet
 - Increasing the passive permeability will likely increase Fmet, but little change with ER
- Repeat the simulations with NumberPGP = 0. This simulates the (complete) inhibition of efflux transporters, and hence, the effect of enzyme-transporter interplay can be measured
 - Without the efflux transporters, Fmet is likely to increase, but ER may increase or decrease
- One can also explore using different affinities, different dose (NumberCompound) or changing the dosing direction (Check the B2ADose box)
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