Multi-Scale modelling of the Bile Acid and Xenobiotic System

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Abstract: *Systems biology has developed considerably in the past decade combining the different disciplines of mathematical modelling, computational simulation and biological experimentation facilitating the quantitative analysis of biological systems. This is often severely hampered by the lack of time-resolved data which ultimately leads to problems in validating any models created. To address the inherent complexity in biological systems, a recent trend in systems biology is exploring multi-scale modelling and simulation methodologies. We consider the Bile Acid and Xenobiotic System (BAXS) as a typical example of a multi-scale system. In the absence of dynamic data from biological experimentation the models we have developed are based on artificial data which enables us to explore multi-scale modelling and validation techniques and the integration of individual models. The outcome of this study will direct further research into multi-scale modelling methodology and ultimately will produce a novel framework for validation in the absence of dynamic data*.

Keywords: Systems biology, multi-scale modelling, simulation, xenobiotics, bile acids.

1. Introduction

The main focus for this research is addressing the inherent complexity in biological systems by exploring multi-scale modelling and simulation methodologies. To facilitate this investigation we model the *bile acid and xenobiotic system* (*BAXS*), a typical example of a multi-scale biological system adopting a multi-scale modelling and simulation approach. The BAXS describes a genetic network that facilitates two distinct but intimately overlapping physiological processes; The enterohepatic circulation and maintenance of bile acid concentrations (Figure 1) and the detoxification and removal from the body of harmful xenobiotic (e.g. drugs, pesticides), and endobiotic compounds (e.g. steroid hormones) [1]. The system involves the coordination of several levels of gene activity, including control of mRNA and protein expression and regulation of metabolising enzyme and transporter protein function in tissues such as liver, intestine/colon and kidney. Bile acids are necessary for the emulsification and absorption of dietary fats and are therefore valuable compounds, however as their build-up can cause harm, their concentrations need to be appropriately regulated and recycled. Similarly there is a requirement for a system that can 'sense' the accumulation of xenobiotic and endobiotic compounds and facilitate their detoxification and removal from the body. The BAXS accomplishes this and maintains enterohepatic circulation (the circulation of biliary acids from the liver, depicted Figure 1) through a complex network of sensors in the form of *nuclear receptors* that function as ligand-activated transcription factors.

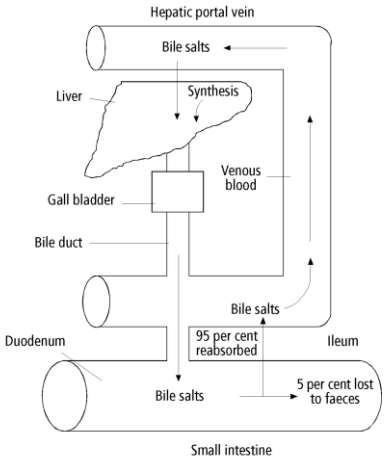


Figure 1. Schematic illustration of enterohepatic circulation.

They serve to detect fluctuations in concentration of many compounds and initiate a physiological response by regulating the BAXS. Transcriptional regulation by nuclear receptors involves both activating and repressive effects upon specific 'sets' of genes. There is considerable overlap exhibited between nuclear receptors in the genes they target and also the ligands that bind to and activate them. It is these factors that contribute to the phenomenon of drug-drug interactions, e.g. between St. John's Wort and Cyclosporine [2] or St. John's Wort and Oral contraceptive [3]. Positive feed-forward and negative feed-back loops can also occur, e.g. within the cholesterol metabolic pathway [4, 5]. Multi-scale modelling of the BAXS will benefit biologists interested in exploring such phenomena. Multi-scale systems biology modelling efforts aim to explore such multi-scale systems quantitatively by means of simulations that integrate several (usually independently developed) single-scale models into a coherent multi-scale model [6]. Our aim is to capture and model separate BAXS processes individually and combine them using a multi-scale modelling approach. For example, in the BAXS the initial stimuli leading to a physiological response would be the binding of a ligand by a

nuclear receptor. The process following the ligand-receptor binding event involves the bound nuclear receptor binding to response elements in the target genes and the cascading effects of increased gene expression that would ensue. Subsequent processes include conjugation and transporter functions [7]. Each single process can be modelled separately regardless of the different scales the may operate in. They can be referred to as separate modalities of biology thus the approach taken is 'multi-modal'. The 'modularity' or multi-biology approach better reflects the way biologists would do experiments, investigating one constituent process at a time, each yielding a separate data set. Single-scale / single-biology models can be built from these experiments and then these individual models can be integrated into a multi-scale/multi-biology model. Each single scale model can then be reverse engineered separately and then integrated with a suitable coupling approach. Alternatively all single scale models can be reverse engineered in a single reverse engineering process however this approach must include the coupling within the reverse engineering phase. Through such experimentation the aim is to address the problems associated with multi-scale modelling and validation, specifically the coupling of processes operating on different scales.

Developing dynamic models of biological process and systems requires dynamic (time-resolved) quantitative data. Such time-series data provides measurements being recorded at certain, pre-defined intervals over a period of time. For many biological systems or processes of interest, sufficient dynamic data required for modelling may not be available [8, 9, 10]. For example, many experimental protocols in biology require the killing of their specimen. This approach precludes the collection of individual-based time series data. Systems biology is still a developing field and current biological experimentation is rapidly changing to produce quantitative data facilitating the development (including validation) of dynamic models. Currently however, for many biological systems of interest, there is insufficient data to develop and validate dynamic models.

2. BAXS processes

Nuclear receptors are a class of proteins found within the interior of cells that are responsible for sensing the presence of steroid and thyroid hormones and certain other molecules. In response, these receptors work in concert with other proteins to regulate the expression of specific genes, thereby controlling the development, homeostasis, and metabolism of the organism. Nuclear receptors have the ability to directly bind to DNA and regulate the expression of adjacent genes. Hence, these receptors are classified as *transcription factors*[1]. The regulation of gene expression by nuclear receptors occurs only when a *ligand* — a molecule that affects the receptor's behavior (i.e., activate or deactivate it) — is present. More specifically, ligand binding to a nuclear receptor results in a conformational change of the receptor molecule complex, which in turn activates the receptor resulting in up-regulation of gene expression. A unique property of nuclear receptors that differentiates them from other types of receptors is their ability to directly interact with and control the expression of genomic DNA. As a consequence, nuclear receptors play a key role in both embryonic development and adult homeostasis.

Our BAXS modelling efforts are directed first at the effects of ritonavir on the metabolism of hyperforin in the liver and the overlap of this process with FXR mediated primary and secondary bile acid metabolism. We refer to this as the *Liver scenario* which is depicted in the diagram of Figure 2. Its main constituent elements and processes are described below.

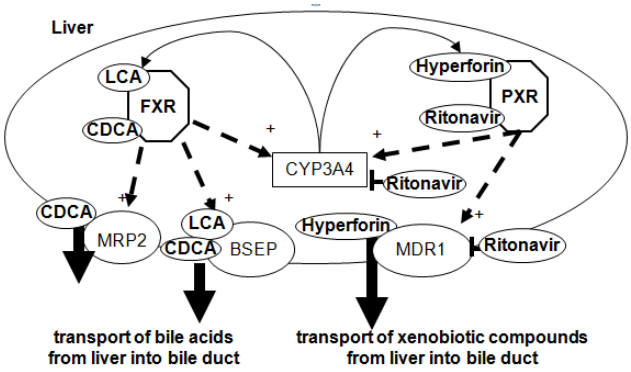


Figure 2. Metabolism of hyperforin and bile acid in liver. *PXR-mediated metabolism of hyperforin in the liver inhibited by ritonavir, FXR mediated bile acid metabolism and the transport process.*

Pregnane X receptor (PXR) is a nuclear receptor highly expressed in the liver encoded by the *NR1I2* (nuclear receptor subfamily 1, group I, member 2) gene. Its primary function is to sense the presence of foreign toxic substances and in response up-regulate the expression of proteins involved in the detoxification and clearance of these substances from the body [11].

Farnesoid X receptor (FXR), a nuclear receptor encoded by the *NR1H4* (nuclear receptor subfamily 1, group H, member 4) gene is also known as the bile acid receptor. It is highly expressed in the liver and its primary function is to sense the presence of bile acids and protect the body from elevated bile acid concentrations [12].

Hyperforin is a herbal antidepressant found in St. John's wort and is an activating ligand for PXR [13]. Activated PXR up-regulates transcription of *CYP3A4* (measured in hours) producing enzymes which metabolise Hyperforin (measured in seconds to minutes) [14]. PXR also targets the gene encoding MDR1 [15], a transporter protein which transports hyperforin from the cell (measured in seconds to minutes).

Ritonavir is a protease inhibitor, often prescribed to HIV patients as part of antiretroviral therapy [16]. HIV protease is an enzyme which cuts the raw material for HIV into specific pieces needed to build a new virus. Protease inhibitors block the protease enzyme preventing it from working, thus

[1] Transcription factors activate or repress the transcription of a gene by controlling the time and rate of transcription of a gene's DNA into RNA.

incomplete, defective copies of HIV are formed which cannot infect cells. Ritonavir is also an activating ligand for PXR [17], however without receptor binding it can repress metabolism and transporter activity induced from transcription of CYP3A4 and MDR1 through competitive inhibition (measured in seconds and minutes). This could lead to a possible build-up of hyperforin in the liver.

The bile acid receptor (BAR), also known as farnesoid X receptor (FXR) is activated by primary and secondary bile acids, lithocholic acid (LCA) and chenodeoxycholic acid (CDCA). It up-regulates transcription of *CYP3A4*, *MRP2* and *BSEP*, the latter two encoding transporter proteins which transport bile acids into the bile duct. The overlap of both processes occurs at the *CYP3A4* gene and several scenarios can be explored. A patient taking hyperforin will have increased expression of *CYP3A4* which may lead to a deficiency in bile acid concentration as this gene produces enzymes which metabolise bile acids. Similarly a patient with high bile acid concentrations may reduce the efficacy of hyperforin (if taken) as transcription of CYP3A4 is increased. If ritonavir is added to this example then bile acids and hyperforin could accumulate to toxic levels in the liver.
A second scenario which will be considered in future work looks at the effects of ritonavir on the metabolism of hyperforin in the intestine and the overlap of this process with VDR-mediated vitamin D metabolism.

3. Multi-scale modelling

Starting with early studies beginning in 1990s [18] multi-scale modelling and simulation has now turned into a focal point of attention across many scientific and engineering disciplines. An increasing number of scientific papers are published, workshops are organized and some specialized journals exist. Communities (ranging from physics and biology to medicine, finance, and engineering) are confronted with the problem of understanding multi-scale systems that are central to their field of study. For instance, the Virtual Physiological Human project [19], funded by the EC, is a good example of a community concerned with multi-scale modelling and simulation of human physiology. The COAST project developed a multi-scale modelling methodology [20] whose basic building blocks comprise single-scale models and their mutual multi-scale couplings. Many, if not all, multi-scale models can be expressed in this general multi-scale modelling framework. In the COAST framework, a multi-scale model can be represented as a directed graph on a *scale separation map* (*SSM*), which is a plot that has the relevant range of scales on its axes (usually space and time, but other quantities are possible). The single-scale models are positioned on the SSM according to their characteristic scales, and the coupling templates are represented as directed edges (Figure 3). While many approaches to systems biology involve single-scale models, there is a growing body of work that aims at modelling of life phenomena across several *scales*. Multi-scale systems biology is concerned with experiments and hypotheses that involve different scales of biological organization from intracellular molecular interactions to cellular behaviour and the behaviour of cell populations (Figure 3). Multi-scale systems biology modelling efforts aim to explore such multi-scale systems quantitatively by means of simulations that incorporate several different simulation techniques because of the different *temporal scales* and *spatial scales* involved [6, 21, 22].

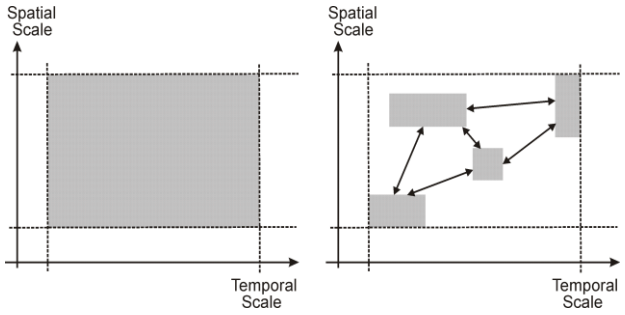
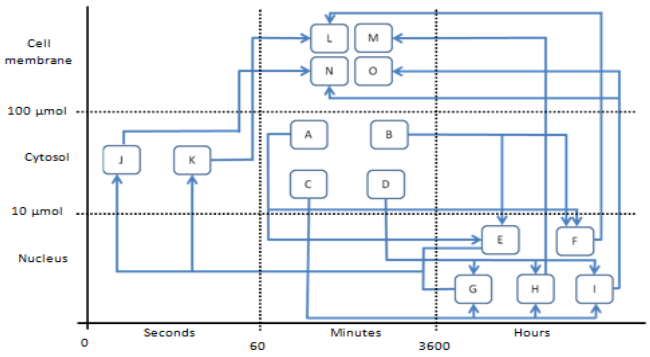


Figure 3. The scale separation map.
Decomposition of a multi-scale system: Left, a multi-scale model spanning many temporal and spatial scales. Right, the resulting decomposed model, consisting of four coupled single scale models.

Qualitative diagrammatic multi-scale models are very common in biomedical research. Ultimately all biological properties on the level of tissues or organs are based on molecular interactions occurring within or on the surface of cells. Biologists frequently describe the hypothetical role a specific molecular mechanism may play in a tissue-level disease by means of a diagram with an arrow connecting molecular entities to a higher scale entities associated with the disease. However, if one wants to subject the proposed causal relationships to a stringent quantitative exploration one needs to transform the knowledge embodied in the arrow-based diagram into a formal description suitable as input for computer simulations. The SSM depicted in Figure 4 represents the Liver BAXS scenario as described above.



Legend:

Ligand/Receptor binding:
A PXR binds hyperforin
B PXR binds ritonavir
C FXR binds LCA
D FXR binds CDCA

Receptor activates gene:
E PXR activates CYP3A4
F PXR activates MDR1
G FXR activates CYP3A4
H FXR activates MRP2
I FXR activates BSEP

Enzyme activity on substrate (inhibited by ritonavir)
J CYP3A4 metabolises LCA
K CYP3A4 metabolises Hyperforin

Transport of substrate from cell (inhibited by ritonavir)
L MDR1 transports metabolised hyperforin to exosol
M MRP2 transports CDCA to exosol
N BSEP transports metabolised LCA to exosol
O BSEP transports metabolised CDCA to exosol

Figure 4. SSM representing the Liver scenario.

Each individual process in this scenario has been identified in terms of the spatial and temporal scales within which they occur. The first group of processes (labelled A to D in the diagram) operate within the cytosol and involve the binding of ligand to nuclear receptor which can be measured on a time scale of minutes. The next group of processes (labelled E to I) take place in the nucleus and result in an increased rate of gene expression. These processes operate on the scale of hours. Processes J and K take place in the cytosol, involve the metabolism of the ligand through increased enzyme activity and include the inhibitory effects of another substrate on the metabolic process through competitive inhibition. These processes are measured on a scale of micro-seconds to seconds.

The processes labelled L to O are localized in and at the cell membrane and involve the transport of metabolized substrates across the membrane out of the cell by transporter proteins. These processes also include competitive inhibition of another substrate. These processes occur over a time scale of minutes. To simplify the modelling approach, the processes are grouped together such that process A represents the binding of ligand and nuclear receptor, process B represents gene expression, process C represents enzyme activity on a substrate, including competitive inhibition, and process D represents activity of transporter proteins as shown inFigure 6. Additionally, the initial models created represent the pathway resulting from PXR activation only. This will be further developed to include the FXR pathway once the modelling techniques have been established.

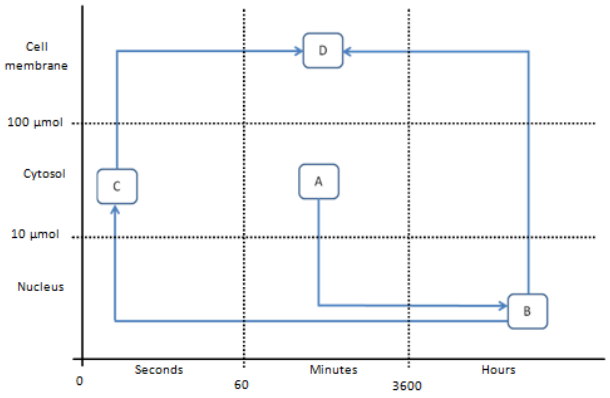
The ligand receptor binding process is governed by mass action kinetic laws [23] which determine the rate at which the overall reaction occurs. The reaction equations below describe how this process occurs and how the kinetic laws are applied.

$$L + R \xrightarrow{k_{on}} LR \qquad \textbf{Eq. 1}$$

$$LR \xrightarrow{k_{off}} L + R \qquad \textbf{Eq. 2}$$

Eq. 1 shows that ligand (L) plus nuclear receptor (R) bind to create the ligand/nuclear receptor complex (LR). The rate at which this occurs is determined by the kinetic constant k_{on} which is the association rate for the ligand binding to the nuclear receptor. This reaction is reversible therefore Eq. 2 shows the dissociation of the ligand/receptor complex into its constituent compounds and the rate is determined by the kinetic constant k_{off} which is the dissociation rate of the bound nuclear receptor complex. The combination of both reactions determines the overall rate of complex formation.

The transactivation process resulting in increased gene expression is triggered by the activated PXR complex resulting from process A (either bound to hyperforin or ritonavir) translocating to the cell nucleus and binding to DNA. Among the target genes are *CYP3A4* which produces the enzyme cytochrome p450, and *MDR1* which produces the transporter protein p-glycoprotein, an ATP binding cassette transporter (ABC-transporter).



Legend:

A	Ligand/Receptor binding.	**C**	Enzyme activity on substrate.
B	Receptor activates gene expression.	**D**	Transport of substrate from cell.

Figure 5. Simplified SSM representing the Liver scenario. *Grouping all similar process types together for modelling purposes.*

The transcription process follows kinetic laws determined by the Hill function for transcriptional activation [24, 25]. Eq. 3 shows the equation determining the overall rate of mRNA production

$$\frac{k_1 [A]^n}{k_m{}^n + [A]^n} \qquad \textbf{Eq. 3}$$

where A denotes the activator (the concentration of the PXR compound), k_1 the maximal transcription rate of the gene, k_m the activation co-efficient and n the Hill coefficient.

As mRNA is produced it translocates to the cytosol and is translated into protein at the ribosome. Eq. 3 shows the equation determining the overall rate mRNA is translated into protein. The rate of this reaction follows the kinetic laws of mass action

$$k_2\,[\text{mRNA}] \qquad \textbf{Eq. 4}$$

where k_2 is the translation rate which represents the number of protein molecules produced per mRNA molecule per unit of time.

The ligand receptor binding model was implemented in COPASI [26], a software tool for simulation and analysis of biochemical networks and their dynamics. The final model forms a mathematical representation of the biological process under study upon which dynamic simulations can be run. Table 1 details the initial concentrations used in the model for ligand-receptor binding. Table 2 shows the reactions between species in the model and the parameter values used. Due to the absence of data from biological experimentation the values used in the model where estimated through a process of trial and error.

Table 1. Initial concentrations for ligand receptor binding model.

Species	Initial concentration (µmol/l)
Hyperforin	600
Ritonavir	500
PXR	10

Table 2. Ligand receptor binding model reactions.

Reaction	Equation	Rate
Ass. of Hyp with PXR	PXR + Hyp → PXR:Hyp	8e-06 l/(µmol*s)
Diss. of Hyp and PXR	PXR:Hyp → PXR + Hyp	6.5e-07 1/s
Ass. of Rit with PXR	PXR + Rit → PXR:Rit	9e-06 l/(µmol*s)
Diss. of Rit and PXR	PXR:Rit → PXR + Rit	7.5e-07 1/s

Ass. = association; Diss. = dissociation;
Hyp = hyperforin; Rit = ritonavir

A simulation was run in COPASI with the duration set to 600 seconds (10 minutes) and interval size at 2 seconds resulting in a dataset with 300 time-steps. Figure 6 shows the resulting graph of plotting the simulated data.

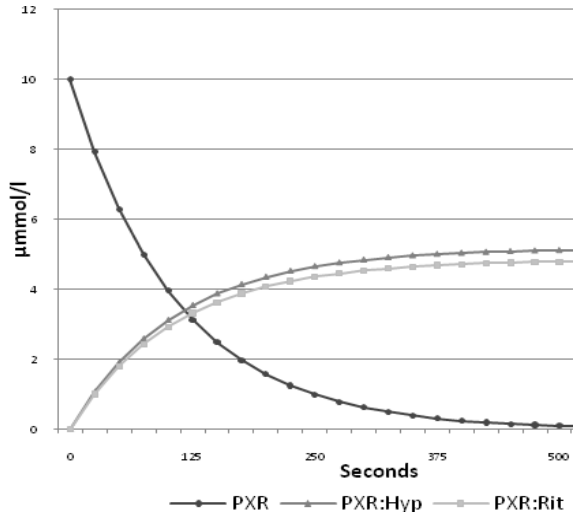


Figure 6. Ligand receptor binding model:
Species concentration (vertical axis) over time (horizontal axis).

A second model was created in COPASI to simulate the reactions involved in process B, which result in activation of gene expression.

Table 3 shows the initial concentrations used in the gene expression model and Table 4 details the reactions rates and parameter values used. The duration for the simulation was set to 100 000 seconds (27.7 hours) with 2500 time steps of 40 seconds each. Figure 7 shows the result of plotting the simulated data. Again, the initial values, rates and parameters have been estimated through a process of trial and error due to the lack of experimental data.

Table 3. Initial concentrations for gene expression model.

Species	Initial concentration (µmol/l)
PXR:Hyp	5.14
PXR:Rit	4.82

Table 4. Gene expression model reactions.

Reaction	Equation	Rates / Parameters
Diss. of PXR:Hyp complex	PXR:Hyp → PXR + Hyp	0.00085 1/s
Diss. of PXR:Rit complex	PXR:Rit → PXR + Rit	0.00095 1/s
Transc of CYP3A4 by PXR:Hyp	→ CYP3A4(m); PXR:Hyp	k1 = 0.003, n = 1, km = 0.5
Transc. of CYP3A4 by PXR:Rit	→ CYP3A4(m); PXR:Rit	k1 = 0.006, n = 1, km = 0.5
Transc. of MDR1 by PXR:Hyp	→ MDR1(m); PXR:Hyp	k1 = 0.005, n = 1, km = 0.5
Transc. of MDR1 by PXR:Rit	→ MDR1(m); PXR:Rit	k1 = 0.007, n = 1, km = 0.5
Transl. of CYP3A4 mRNA	CYP3A4(m) → CYP3A4	k2 = 2.4e-05 1/s, d2 = 1e-05 1/s
Transl. of MDR1 mRNA	MDR1(m) → MDR1	k2 = 2.7e-05 1/s, d2 = 1e-05 1/s

Diss. = dissociation; Transc. = transcription;
Transl. = translation; Hyp = hyperforin; Rit = ritonavir

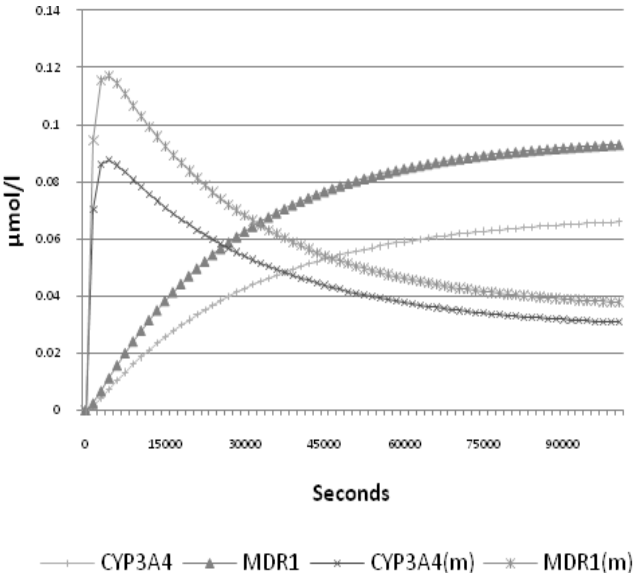


Figure 7. Gene expression model:
Species concentration (vertical axis) over time (horizontal axis).

4. Results

The ligand-receptor binding model indicates a steady increase in bound PXR correlated to a steady decrease in available (unbound) PXR. The initial concentrations of ritonavir and hyperforin decrease steadily (not shown) relative to the accumulation of bound PXR. The entire process is modelled over 600 seconds and reaches a steady state after approximately 500 seconds where the rate of formation of bound PXR begins to level out. The data indicates that after 600 seconds the concentration of PXR bound to ritonavir is 4.82 µmol/l and the concentration of PXR bound to hyperforin is 5.14 µmol/l. To initiate the transcription process only a minimum concentration of activated PXR is required. Process B can therefore start

before process A has finished therefore the processes are not necessarily sequential in nature.

An exchange of data from process A to B is required during the simulation of process A at predefined intervals. The gene expression model indicates a sharp increase in mRNA production peaking at approximately 5000 seconds (approximately 1.5 hours) after which there is a gradual decline.

The translation of mRNA into protein is indicated as a gradual increase in MDR1 and CYP3A4 concentrations which approach steady state at approximately 100 000 seconds (27.7 hours).

The process of enzyme activity on a substrate (process C) is yet to be modelled, however it is dependent on the concentration of the enzymes produced in the gene expression process (process B). As with the integration of processes A and B the relationship between processes B and C is not necessarily sequential. A minimum concentration of enzyme is required to initiate the metabolic process, the rate of which increases as enzyme concentration increases. Each model has been determined as the trigger for the subsequent process, however the processes are not sequential, therefore the integration or 'coupling' of models needs to be studied in more detail. This forms one of the major research areas for this project.

5. Model integration

To investigate how separate individual processes operating on different scales interact with each other a stock and flow diagram was created in Stella[2] for the processes under study (Figure 8). The stock and flow diagram treats the components of the model as stocks, e.g. 'Le' is a stock of ligand outside the cell. The flows represent the rate of change of the stock, either localization or change of state, e.g. 'Le' flows into the cell at a defined rate and accumulates as 'L' which represents the stock of ligand in the cell. The flow from the ligand stock (L) combines with the flow from receptor stock (R) to accumulate as bound ligand receptor stock (LR). This stock has a positive effect on the flows resulting in enzyme production (E1 and E2) represented by the arcs connecting the stock to the flows. Enzyme 1 stock (E1) has a positive effect on the flow of ligand (L) to its metabolised form (L\OH) and enzyme 2 (E2) has a positive effect on the flow of metabolised ligand (L\OH) out of the cell. Finally the stock of inhibitor (I) has a negative effect on the flow of ligand to metabolised ligand and the flow of metabolised ligand out of the cell. By studying the model in terms of stocks and flows it is easy to visualise the interactions in the model as an exchange of stocks. In terms of *coupling* multi-scale models the exchange of data must therefore represent a concentration of a component or components in the individual processes. For example the integration of processes A and B, ligand binding and gene expression, is an exchange of data representing the concentration of activated PXR, the interaction of processes B and C, gene expression and enzyme activity, is an exchange of data representing enzyme concentrations. As the processes are not necessarily sequential, exchange of data has to occur at predefined time steps within the model operating on the smaller scale.

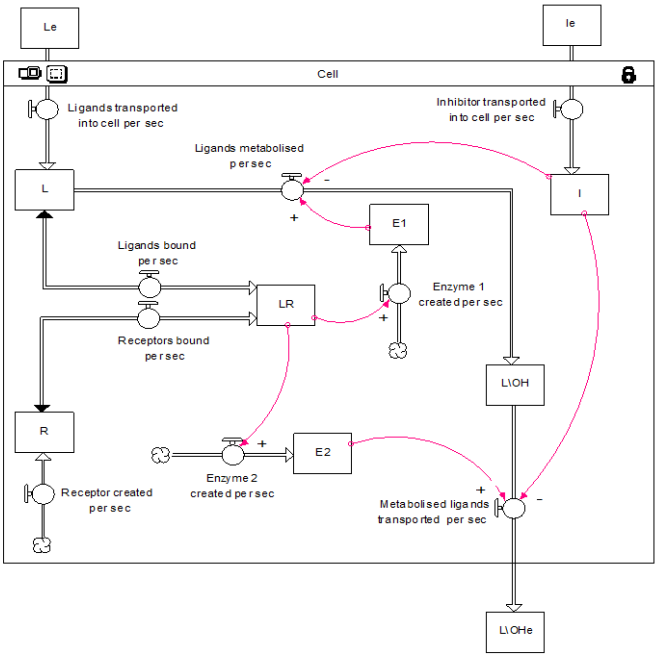


Figure 8. Stock and flow diagram.
Representing the metabolism of hyperforin inhibited by ritonavir in the liver.

E.g. the duration of the ligand binding model is 600 seconds with interval sizes of 2 seconds whereas the duration of the gene expression model is 100 000 seconds with time intervals of 40 seconds. This would mean that for every 20 time steps of process A an exchange of data can occur with process B. This forms the basis for developing methodology for coupling multi-scale processes and allows us to explore this problem further. The development of a 'data generator' using Java has begun which will be able to open two separate instances of Copasi and run two simulations together. It will also be able to interrupt the simulations at specific time intervals and facilitate the exchange of data in either direction as required.

6. Discussion

This study leads us to suggest the most suitable approach in multi-scale modelling and simulation is to deconstruct the entire system into individual processes and model each separately. The *coupling* of models can then be explored in more detail. We suggest the integration or *coupling* of separate models involves an exchange of data representing a stock or concentration of a component within the individual models. The development of a data generator in Java allows this integration of models to be further explored and developed to include other modelling methodologies. This research project has also raised several issues which require further investigation and prompt further research in the fields

[2] STELLA is a general-purpose modelling and simulation tool of isee systems: www.iseesystems.com.

of biology and systems biology. The models created in Copasi use artificial data to quantify the kinetic rates of reactions within the processes under study. This project would benefit greatly if biological experimentation in this area could provide real data upon which to validate the models. Further models will be developed to capture the additional processes detailed in the SSM and the 'data generator' will be implemented to explore the coupling of these separate processes. Ultimately the 'data generator' will be developed to explore the integration of different spatial scales in biology, including the integration of models using different methodologies e.g. cellular automata, agent based modelling. Multi-scale modelling and simulation is more complex than single-scale modelling and simulation. On the biology side it involves different temporal and spatial scales as well as different types of biological process and entities. On the mathematical side, different methods may be used to model the different sub-models of a multi-scale model. Furthermore, specific methods may be used to couple the different sub-models. On the computational side many intricate issues arise.

A new EC-funded project with University of Ulster participation aims to develop computational strategies, software and services for distributed multi-scale simulations across disciplines, exploiting existing and evolving European e-infrastructure [27]. Our preliminary literature research on evaluation and validation of multi-scale modelling and simulation in biology shows that there is a lack of suitable detailed work in this area. This and the lack of suitable dynamic data for modelling of the BAXS has prompted us to pursue the development of a testing environment which would allow us (1) To generate unlimited dynamic data related to the BAXS, (2) develop and study multi-scale modelling and simulation approaches for the BAXS, and (3) study, apply and develop validation techniques for multi-scale modelling and simulation in systems biology. The basic idea of this testing environment is based on the Turing-like test for biology [28].

7. References

[1] Kliewer, S.A. & Willson, T.M. 2002, "Regulation of xenobiotic and bile acid metabolism by the nuclear pregnane X receptor", *Journal of Lipid Research,* vol. 43, no. 3, pp. 359-364.

[2] Barone, G., Gurley, B., Ketel, B., Lightfoot, M. & Abul-Ezz, S. 2000, "Drug interaction between St. John's wort and cyclosporine", *The Annals of Pharmacotherapy,* vol. 34, no. 9, pp. 1013-1016.

[3] Hall, S.D., Wang, Z., Huang, S., Hamman, M.A., Vasavada, N., Adigun, A.Q., Hilligoss, J.K., Miller, M. & Gorski, J.C. 2003, "The interaction between St John's wort and an oral contraceptive[ast]", *Clinical Pharmacology and Therapeutics,* vol. 74, no. 6, pp. 525-535.

[4] Eloranta, J.J. & Kullak-Ublick, G.A. 2005, "Coordinate transcriptional regulation of bile acid homeostasis and drug metabolism", *Archives of Biochemistry and Biophysics,* vol. 433, no. 2, pp. 397-412.

[5] Goodwin, B., Redinbo, M.R. & Kliewer, S.A. 2002, "Regulation of CYP3A gene transcription by the pregnane X receptor", *Annual Review of Pharmacology and Toxicology,* vol. 42, no. 1, pp. 1-23.

[6] Dada, J.O. & Mendes, P. 2011, "Multi-scale modelling and simulation in systems biology", *Integr.Biol.,* vol. 3, no. 2, pp. 86-96.

[7] Stieger, B. & Meier, P.J. 1998, "Bile acid and xenobiotic transporters in liver", *Current Opinion in Cell Biology,* vol. 10, no. 4, pp. 462-467.

[8] Mendoza, L. & Xenarios, I. 2006, "A method for the generation of standardized qualitative dynamical systems of regulatory networks", *Theor Biol Med Model,* vol. 3, pp. 13.

[9] Kitano, H. 2002, "Computational systems biology", *Nature,* vol. 420, no. 6912, pp. 206-10.

[10] Endy, D. & Brent, R. 2001, "Modelling cellular behaviour", *Nature,* vol. 409, no. 6818, pp. 391-395.

[11] Kliewer, S.A. 2003, "The Nuclear Pregnane X Receptor Regulates Xenobiotic Detoxification", *The Journal of Nutrition,* vol. 133, no. 7, pp. 2444S-2447S.

[12] Chawla, A., Repa, J.J., Evans, R.M. & Mangelsdorf, D.J. 2001, "Nuclear Receptors and Lipid Physiology: Opening the X-Files", *Science,* vol. 294, no. 5548, pp. 1866-1870.

[13] Chang, T. 2009, "Activation of Pregnane X Receptor (PXR) and Constitutive Androstane Receptor (CAR) by Herbal Medicines", *The AAPS Journal,* vol. 11, no. 3, pp. 590-601.

[14] Lemaire, G., Mnif, W., Pascussi, J., Pillon, A., Rabenoelina, F., Fenet, H., Gomez, E., Casellas, C., Nicolas, J., Cavailles, V., Duchesne, M. & Balaguer, P. 2006, "Identification of New Human Pregnane X Receptor Ligands among Pesticides Using a Stable Reporter Cell System", *Toxicological Sciences,* vol. 91, no. 2, pp. 501-509.

[15] Lin, Y.S., Yasuda, K., Assem, M., Cline, C., Barber, J., Li, C., Kholodovych, V., Ai, N., Chen, J.D., Welsh, W.J., Ekins, S. & Schuetz, E.G. 2009, "The Major Human Pregnane X Receptor (PXR) Splice Variant, PXR.2, Exhibits Significantly Diminished Ligand-Activated Transcriptional Regulation", *Drug Metabolism and Disposition,* vol. 37, no. 6, pp. 1295-1304.

[16] Koudriakova, T., Iatsimirskaia, E., Utkin, I., Gangl, E., Vouros, P., et al., 1998, "Metabolism of the Human Immunodeficiency Virus Protease Inhibitors Indinavir and Ritonavir by Human Intestinal Microsomes and Expressed Cytochrome P4503A4/3A5: Mechanism-Based Inactivation of Cytochrome P4503A by Ritonavir", *Drug Metabolism and Disposition,* vol. 26, no. 6, pp. 552-561.

[17] Willson, T.M. & Kliewer, S.A. 2002, "Pxr, car and drug metabolism", *Nat Rev Drug Discov,* vol. 1, no. 4, pp. 259-266.

[18] Broughton, J.Q., Abraham, F.F., Bernstein, N. & Kaxiras, E. 1999, "Concurrent coupling of length scales: Methodology and = application", *Phys.Rev.B,* vol. 60, no. 4, pp. 2391-2403.

[19] VPHnoe 2010, *, Virtual Physiological Human network of excellence*. Available: http://www.vph-noe.eu/ [2011, 3/1/2011] .

[20] Hoekstra, A.G., Lorenz, E., Falcone, J. & et al. 2007, "Towards a complex automata framework for multi-scale modeling", *International Journal for Multiscale Computational Engineering,* vol. 5, pp. 491-502.

[21] Meier-Schellersheim, M., Fraser, I.D.C. & Klauschen, F. 2009, "Multiscale modeling for biologists", *Wiley Interdisciplinary Reviews: Systems Biology and Medicine,* vol. 1, no. 1, pp. 4-14.

[22] Schnell, S., Grima, R. & Maini, P.K. 2007, "Multiscale modeling in biology", *American Scientist,* vol. 95, no. 1, pp. 134-142.

[23] Lees, P., Cunningham, F.M. & Elliott, J. 2004, "Principles of pharmacodynamics and their applications in veterinary pharmacology", *Journal of Veterinary Pharmacology and Therapeutics,* vol. 27, no. 6, pp. 397-414

[24] Kim, H.D. & O'Shea, E.K. 2008, "A quantitative model of transcription factor-activated gene expression", *Nat Struct Mol Biol,* vol. 15, no. 11, pp. 1192-1198

[25] Imperial College 2008, *Synthetic Biology*. Available: http://openwetware.org/wiki/Imperial_College/Courses [2011, 2/23/2011]

[26] Hoops, S., Sahle, S., Gauges, R., Lee, C., Pahle, J., Simus, N., Singhal, M., Xu, L., Mendes, P. & Kummer, U. 2006, "COPASI - a COmplex PAthway SImulator", *Bioinformatics,* vol. 22, no. 24, pp. 3067-3074.

[27] EU MAPPER Project 2010, *, MAPPER Project*. Available: http://www.mapper-project.eu/ [2011, 3/3/2011] .

[28] Harel, D. 2005, "A Turing-like test for biological modeling", *Nat Biotech,* vol. 23, no. 4, pp. 495-496.