Quasi Steady State Petri Net (QSSPN)

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From 2016: Head of Systems Modelling, Simcyp, a Certara Company, Sheffield, UK
Outline

• Motivation: Computer simulation of molecular cell biology
• Motivation: Large mechanistic models in Large Pharma
• Flux Balance Analysis of Genome Scale Metabolic Networks
• Quasi-Steady State Petri Nets (QSSPN)
Statistical inference of genotype-phenotype relationship.

We can determine any genotype of interest, including full genome sequence of an individual.

Genotype-phenotype relationship is fundamental problem in basic and applied science. Knowledge will revolutionise medicine and biotechnology.

Currently, statistical inference is attractive approach to study genotype-phenotype relationship.
Statistical inference of genotype-phenotype relationship.

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Genotype-phenotype relationship is fundamental problem in basic and applied science. Knowledge will revolutionise medicine and biotechnology.

Currently, statistical inference is attractive approach to study genotype-phenotype relationship.

A meta-analysis of 87,040 individuals identifies 23 new susceptibility loci for prostate cancer

What molecular/physiological mechanisms associate these 23 genes with cancer? How complex genotype-environment-phenotype interactions could be studied with GWAS approach?
Mechanistic computer simulation of genotype-phenotype relationship.

BIBLIOME* OF MOLECULAR BIOLOGY

*Formerly known as library

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Mechanistic computer simulation of genotype-phenotype relationship.
Physiologically Based Pharmacokinetics (PBPK) – whole system mechanistic simulation routinely used in Large Pharma.

Non-eliminating tissues:

\[
\frac{dC_T}{dt} = \frac{Q_T C_A}{V_T} \frac{Q_T C_{v,T}}{V_T}
\]

Eliminating tissues (liver, kidney):

\[
\frac{dC_T}{dt} = \frac{Q_T C_A}{V_T} \frac{Q_T C_{v,T}}{V_T} \frac{Cl_{\text{int}} C_{v,uT}}{V_T}
\]

where:

- \(Q\)  Blood flow [L/h]
- \(V\)  Volume [L/h]
- \(T\)  Tissue
- \(A,v,u\) Arterial, venous, unbound

\[
C_{v,T} = \frac{K_p}{B : P}
\]

- \(B : P\) Blood/plasma partition coefficient
- \(K_p\) Tissue/plasma partition coefficient
- \(Cl_{\text{int}}\) Intrinsic clearance
Physiologically Based Pharmacokinetics (PBPK) – whole system mechanistic simulation routinely used in Large Pharma.

Simulation of drug concentration in Heart:
Physiologically Based Pharmacokinetics (PBPK) – whole system mechanistic simulation routinely used in Large Pharma.
PBPK: – whole system scale, bottom-up, literature-based mechanistic simulation routinely used in Large Pharma.

Permeability-limited models are available for the intestine, liver, kidney, brain, and lung.
Example drug label claim based on PBPK simulation.

HIGHLIGHTS OF PRESCRIBING INFORMATION
These highlights do not include all the information needed to use IMBRUVICA safely and effectively. See full prescribing information for IMBRUVICA.
IMBRUVICA® (ibrutinib) capsules, for oral use
Initial U.S. Approval: 2013

Drug Interactions

Coadministration of Ibrutinib with CYP3A Inhibitors

In a sequential design trial of 18 healthy, fasted volunteers, a single dose of 120 mg of IMBRUVICA was administered alone on Day 1 and a single dose of 40 mg of IMBRUVICA was administered on Day 7 in combination with 400 mg of ketoconazole (given daily on Days 4 - 9). Ketoconazole increased ibrutinib dose-normalized C\text{max} and AUC 29-fold and 24-fold, respectively. Simulations using fasted conditions indicate that moderate CYP3A inhibitors diltiazem and erythromycin may increase AUC of ibrutinib by 5- to 8-fold.

Coadministration of Ibrutinib with CYP3A Inducers

PK data from a dedicated drug interaction trial showed that rifampin (a strong CYP3A inducer) decreases ibrutinib C\text{max} and AUC by more than 13- and 10-fold. Simulations using PBPK suggested that a moderate CYP3A inducer (efavirenz) may decrease the AUC of ibrutinib by up to 3-fold.
Almost 100 label claims informed by PBPK, including DDI, absorption, ethnic bridging, formulation
We can determine full genome sequence of an individual. Genomics has entered clinical research and healthcare (UK NHS 100K genomes project).

Currently, Simcyp simulator can mechanistically account for polymorphism of only about 20 genes (drug metabolism and drug transporters).

Bridging the gap between genomics and physiology through mechanistic modelling of “intracellular space” is a major challenge and solutions will revolutionise model-based drug development.
Quantitative simulation of molecular network dynamics

Quantitative simulation of molecular network dynamics


Propensity: $c_9 \#\text{mRNAKin}$
$c_9 = 10^{-4} \, 1/s$
Molecular networks are commonly represented as bipartite graphs even if it is not referred to as a Petri Net. SBGN and CellDesigner (above) as well as Matlab Simbiology (right) are good examples.
Mechanistic simulation of the relationship between genotype and metabolic phenotype with Flux Balance Analysis (FBA) of Genome Scale Metabolic Network.

Nutrients available in cell environment (external metabolites)

Maximal possible flux through selected reaction in the system (objective function).

**ASSUMPTION:** Internal metabolites are at steady state.
**Flux Balance Analysis – a constraint based approach**

Variables of the model are reaction fluxes at steady state $F_1, \ldots, F_8$. Stoichiometric matrix $S$ represents contribution of metabolites (rows) to reactions (columns). The unique maximal value of any linear combination of fluxes can be calculated by linear programming. Maximal objective value can be achieved by many flux distributions (solutions). **FBA generates insightful qualitative predictions based on network connectivity alone, but quantitative information can be used to constraint solution space.**

From Orth, Thiele, Palsson Nature Biotechnology 2010, 28:245

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Computer simulation of genotype-phenotype relationship.


GSMN-TB: a web-based genome-scale network model of Mycobacterium tuberculosis metabolism

Research

Highly accessed Open Access

Genome Biology 2007, 106 citations.
Reconstruction and Simulation of Genome Scale Metabolic Networks @Surrey

Lipid metabolism and Type VII secretion systems dominate the genome scale virulence profile of Mycobacterium tuberculosis in human dendritic cells

Tom A Mendum, Huijal Wu, Andrzej M Kierzek and Graham R Stewart

Differential Producibility Analysis (DPA) of Transcriptomic Data with Metabolic Networks: Deconstructing the Metabolic Response of M. tuberculosis

Bhushan K. Bonde, Dany J. V. Beste, Emma Laing, Andrzej M. Kierzek, Johnjoe McFadden*

Microbial Sciences Division, Faculty of Health and Medical Sciences, University of Surrey, Guildford, United Kingdom

Selection of objective function in genome scale flux balance analysis for process feed development in antibiotic production

Chiraphan Khannapho*, Hongjuan Zhao, Bhushan K. Bonde, Andrzej M. Kierzek, Claudio A. Avignone-Rossa, Michael E. Bushell*

Antimicrobial Science Division, Faculty of Health and Medical Sciences, University of Surrey, Guildford, Surrey GU2 7XH, UK

Interrogation of global mutagenesis data with a genome scale model of Neisseria meningitidis to assess gene fitness in vitro and in sera

Tom A Mendum, Jane Newcombe, Ahmad A Mannan, Andrzej M Kierzek and Johnjoe McFadden*

GSMN-TB: a web-based genome-scale network model of Mycobacterium tuberculosis metabolism


* These authors contributed equally to this work.

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Genome Biology 2007, 8(R89) (doi:10.1186/gb-2007-8-8-r89)

Received: 23 January 2007
Revised: 16 April 2007
Accepted: 23 May 2007
Mechanistic interpretation of transcriptome data.

Mechanistic interpretation of omics data in JyMet GUI and sfba command line tool.

Large number of ~omics data analysis approaches available through Graphics User Interface: iMAT, GIMME, GIM3E, DPA, Fast iMAT, GNI

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Generation of 2,000 breast cancer metabolic landscapes reveals a poor prognosis group with active serotonin production

Vytautas Leoncikas¹, Huihai Wu¹, Lara T. Ward², Andrzej M. Kierzek¹,* & Nick J. Plant¹,*
Summary

The genomic and transcriptomic architecture of 2,000 breast tumours reveals novel subgroups

Christina Curtis1,2,*, Sobrab P. Shah1,2,*, Suat-Fueng Chin1,2,*, Golikha Turgayev1,2,*, Oscar M. Rosell1,2, Mark J. Dunning2, Doug Speed2,*, Andy G. Lynch2,*, Sharmistha Samanta2,*, Yinying Yuan2,*, Stefan Gräf2,*, Gaven H2, Gudmundur Hafstein2, Ali Bashashati2, Robin Russel2, Steven McKinney1,*, METABRIC Group1, Anita Langerod2, Andrew Green1, Elena Provenzano1, Gordon Wishart3, Sarah Fisher4, Peter Watson4,*, Florian Markowitz2,*, Leigh Murphy2,*, Ian Ellis1, Annie Purushotham7,*, Anne-Line Berrevozet-Dub6,*, James D. Brenton2,*, Simon Tavare1,2,*, Carlos Caldas1,2,3,4,5 & Samuel Aparicio1,6


A community-driven global reconstruction of human metabolism

Ines Thiel2,5,*, Neil Swainston3,4,*, Ronan M T Fleming1,*, Andreas Hoppe5, Swagatika Sahoo1, Makke K. Aurich1, Hulda Haraldsdottir1, Monica L Mo1, Ottar Bølviken1, Miranda D Stobbe4,*, Stefan G Thorleifsson1, Rasmus Agren6, Christian Bölling2, Sergio Bordel1,*, Arvind K Chavali1, Paul Dobson2, Warwick B Dunn3,*, Lukas Endler1, David Hala5, Michael Huckle3,*, Duncan Hedd2, Daniel Jameson5,*, Neerma Jammidi1, Jon J Jonsson1, Nick Jesty2,*, Sarah Kroting1,*, Inteswat Noksawan3,*, Nicolas Le Novère1,1,*, Naglis Malyš4,*,*,*, Alexander Mazei5,*, Jason A Papin1,*, Nathan D Price1,*, Evgeni Selkov5,*, Martin I Sigurdsson1, Evangelos Simonoudis2,*, Nikolaus Sommerschein1,*, Kieran Smallbone1,5,*, Anatoly Serekin1,5,*, Johannes H G M van Beek1,2,5, Dieter Weichart1,2,*, Igor Goryanin1,9,*, Jens Nielsen1,*, Hans V Westerhoff1,2,5,9, Douglas B Kell1,2,*, Pedro Mendes1,1,*,* & Berthold O Palsson1,2

Mechanistic model-based meta-analysis of clinical transcriptomes to stratify disease progression in individual patients.

**METABRICK transcriptome arrays, 2000 individual tumours**

- Array data pre-processing and classification of transcripts to absent (-1) and present (0) based on detection p-values.
- Classification of GSMN reactions to absent (-1) and present (0) based on GSMN gene-reaction rules and transcript classification.
- Optimisation of congruency between GSMN constraints and transcriptome-based reaction classification.
- Determination of metabolic landscape by assigning non-active (-1) or active (0) state to each GSMN reaction based on Flux Variability Analysis ranges.

**Recon 2, Genome Scale Metabolic Network (GSMN), 7440 reactions**

- K-means clustering and comparison with survival data.
- 2000 personalised metabolic metabolic landscapes

---

Potential DDI between chemotherapy and SSRI antidepressants.
Quasi-steady state metabolic fluxes.

The timescale separation between gene regulation and metabolism justifies Quasi-Steady State assumption:

**Following the change of gene expression state metabolism quickly reaches state where metabolic flux is constant and metabolite concentrations are balanced.**

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**Metabolism:** COPASI example file YeastGlycolysis


**Gene regulation:** COPASI example file NF-kappaB

Systems biology

QSSPN: dynamic simulation of molecular interaction networks describing gene regulation, signalling and whole-cell metabolism in human cells

Ciarán P. Fisher, Nicholas J. Plant, J. Bernadette Moore and Andrzej M. Kierzek*

Faculty of Health and Medical Sciences, School of Biosciences and Medicine, University of Surrey, Guildford, Surrey GU2 7XH, UK

Associate Editor: Igor Jurisica

2. Slow, dynamic, regulatory processes are modelled by Petri Net

3. For each state of regulatory network fast metabolic reactions are assumed to be at steady state.

4. **Constraint places** set flux bounds of FBA fluxes. **Objective places** execute FBA evaluation of objective functions.

Fisher, Plant, Moore, Kierzek Bioinformatics 29, 3181-3190 (2013)
Propensity function and transition types.

To allow formulation of general rules involving token number thresholds, we define transition propensity:

$$P_t = c_t \prod_{i=1}^{N} \mu_i(x_i)$$

where $P_t$ is a propensity function of transition $t$, $c_t$ is a rate constant, $N$ is the number of pre-places of transition $t$, $x_i$ is the number of tokens at preplace $i$ and $\mu_i$ is the pre-place activity in the transition depending on the pre-place state. The activity function is a look-up table of $T$ thresholds $t_i$ and activities $a_i$ allowing general definition of the pre-place contribution to the transition propensity.

$$\mu(x) = \begin{cases} 
  x \in [t_1, t_2), & \mu(x) = a_1 \\
  x \in [t_i, t_{i+1}), & \mu(x) = a_i \\
  \ldots \\
  x \in [t_{T-1}, t_T), & \mu(x) = a_T 
\end{cases}$$

In new version of the software any arithmetic expression involving pre-place states $x_i$ can also be used to calculate propensity $P_t$.

The interpretation of a transition propensity during simulations is dependent on the transition class:

**Stochastic transition** - propensity is interpreted as the probability density of the transition firing in the next, infinitesimally small, time step. We allow stochastic transitions to be delayed.

**Continuous transitions** – propensity is interpreted as reaction rate.

**Immediate transition** - fires once whenever its propensity function is different than 0.

In new version of the QSSPN software the following transition classes were added:

**Reset transition** – sets state of post places to the value specified by arithmetic expression involving pre-place states $x_i$.

**Flux transition** has only one pre-place which has to be objective node. It resets state of one post-place to one of FBA solution fluxes.
QSSPN simulation algorithm.

`setQSSFbounds()` - This function sets the bounds of fluxes in the quasi-steady state flux (QSSF) part of the model according to the state of the constraint node.

`evaluateObjective()` - This function uses Flux Balance Analysis to evaluate the objective function specified by a particular objective node. The objective function is specified as the name of the flux or the name of the metabolite in the QSSF network. If the objective is specified as the flux the linear programming (LP) maximises value of this flux. If the objective is specified as a metabolite, the sum of fluxes producing this metabolite is maximised.

`updateObjectiveNode()` - This function sets the state of a particular objective node according to the objective function value, thus feeding back information about steady state metabolic capabilities to the dynamic part of the model.

`fireDeterministicTransitions(Δt)` - Each immediate transition for which propensity function is greater than 0 is fired once. In recent version of the software adaptive timestep Euler algorithm is used to simulate state change of places connected to continuous transitions within Δt. All node state updates within this function are executed synchronously.

`fireDelayedTransition(t, t_d)` - This function returns a Boolean value; the function checks if there are any delayed transitions to be fired in the time interval (t, t+t_d). If there are no delayed transitions scheduled to fire the function returns FALSE. Otherwise, it fires one delayed transition and sets simulation time t to the time t_s at which this transition has been scheduled. The Δt parameter is then set to t_s – t. If there are multiple delayed transitions set to fire in the time interval (t, t+t_d) the transition that is scheduled at the earliest time is fired. After firing the transition the function returns TRUE.

`scheduleDelayedTransition(m)` - If the stochastic transition m selected to be fired has delay time t_d greater than 0 this function adds the transition to the list of delayed transitions to be fired. The transition is scheduled to fire at time t_s = t + t_d.
QSSPN = HPN + FBA
Qualitative gene expression model

Fisher, Plant, Moore, Kierzek Bioinformatics 29, 3181-3190 (2013)
The model of bile acid homeostasis in human hepatocyte.

HepatoNet1, Gille et al. Mol. Syst. Biol, 2010

Fisher, Plant, Moore, Kierzek Bioinformatics 29, 3181-3190 (2013)
Fraction of trajectories exhibiting dynamic qualitative behaviour of interest has been compared with experimental data of Song and Chiang, Hepatology 2009.
**Supplementary Table 4.16.** Comparison of behaviours exhibited by experimental data and simulation results.

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Experimental data</th>
<th>Experimental behaviour</th>
<th>Simulation behaviour</th>
</tr>
</thead>
<tbody>
<tr>
<td>FGF19 transcript</td>
<td>Relative amount after treatment with GW4064 for 0, 0.6, 1, 3, 6, 24 hours (Section 4.2.1).</td>
<td>activation</td>
<td>activation</td>
</tr>
<tr>
<td>CYP7A1 transcript</td>
<td>Relative amount after treatment with GW4064 for 0, 0.6, 1, 3, 6, 24 hours (Section 4.2.2).</td>
<td>inhibition</td>
<td>inhibition</td>
</tr>
<tr>
<td>SHP transcript</td>
<td>Relative amount after treatment with GW4064 for 0, 0.6, 1, 3, 6, 24 hours (Section 4.2.3).</td>
<td>burst</td>
<td>burst</td>
</tr>
<tr>
<td>HNF4α transcript</td>
<td>Relative amount after treatment with GW4064 for 0, 0.6, 1, 3, 6, 24 hours (Section 4.2.4).</td>
<td>constant</td>
<td>constant</td>
</tr>
<tr>
<td>CYP7A1 transcript</td>
<td>Relative amount after treatment with FGF19 for 0, 0.6, 1, 3, 6, 24, 48 hours (Section 4.2.5).</td>
<td>inhibition</td>
<td>inhibition</td>
</tr>
<tr>
<td>SHP transcript</td>
<td>Relative amount after treatment with FGF19 for 0, 0.6, 1, 3, 6, 24, 48 hours (Section 4.2.6).</td>
<td>burst</td>
<td>burst</td>
</tr>
<tr>
<td>CYP7A1 transcript</td>
<td>Relative amount with respect to untreated control after treatment with FGF19 (Section 4.2.7).</td>
<td>decrease</td>
<td>decrease</td>
</tr>
<tr>
<td>CYP7A1 transcript</td>
<td>Relative amount with respect to untreated control after treatment with SHP siRNA (Section 4.2.8).</td>
<td>increase</td>
<td>increase</td>
</tr>
<tr>
<td>CYP7A1 transcript</td>
<td>Relative amount with respect to untreated control after treatment with SHP siRNA and FGF19 (Section 4.2.9).</td>
<td>decrease</td>
<td>decrease</td>
</tr>
<tr>
<td>SHP transcript</td>
<td>Relative amount with respect to untreated control after treatment with FGF19 (Section 4.2.10).</td>
<td>equal</td>
<td>equal</td>
</tr>
<tr>
<td>CYP7A1 transcript</td>
<td>Relative amount with respect to GW4064 treatment after FGF19 antibody treatment (Section 4.2.11).</td>
<td>increase</td>
<td>equal</td>
</tr>
<tr>
<td>CYP7A1 transcript</td>
<td>Relative amount with respect to GW4064 treatment after FGFR4 siRNA transcript (Section 4.2.12).</td>
<td>increase</td>
<td>equal</td>
</tr>
<tr>
<td>Chenodiol</td>
<td>Physiological response to rise of cholesterol. (Section 4.2.13).</td>
<td>burst</td>
<td>burst</td>
</tr>
<tr>
<td>Cholate</td>
<td>Physiological response to rise of cholesterol. (Section 4.2.13).</td>
<td>burst</td>
<td>burst</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Metric</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of True Positive predictions (TP)</td>
<td>12</td>
</tr>
<tr>
<td>Number of True Negative predictions (TN)</td>
<td>42</td>
</tr>
<tr>
<td>Number of False Positive predictions (FP)</td>
<td>2</td>
</tr>
<tr>
<td>Number of False Negative predictions (FN)</td>
<td>2</td>
</tr>
<tr>
<td>Matthews Correlation Coefficient (MCC)</td>
<td>0.812</td>
</tr>
</tbody>
</table>

Fisher, Plant, Moore, Kierzek Bioinformatics 29, 3181-3190 (2013)  
www.surrey.ac.uk
Mechanistic simulation of genotype-phenotype relationship.

Colour spectrum represents fraction of trajectories exhibiting behaviour of interest. Increasing colour intensity represents higher fraction of trajectories. The knockdown/behaviour pairs where fractions are within 95% CIs of WT are assigned the same colour as WT.
Model of signalling pathways regulating translation in mammalian cells.

Work of David Taylor.

300 molecular species, 241 interactions based on 1,158 literature references.

Reachability of molecular targets under experimental conditions (input) studied by Statistical Model Checking.

Colour reflect the change in the number of transition firings resulting from application of Ku0063794 inhibitor of mTOR pathway.

Simulation led to hypotheses that were experimentally validated.

Manuscript in preparation.

Significant predictive power evaluated by comparison with comprehensive benchmark of literature data on signalling network inhibitors (MCC = 0.45)
Formal verification of qualitative QSSPN model.

All possible sequences of transitions (reachability graph) are examined to prove that certain behaviour is not feasible.


www.surrey.ac.uk
Multi-scale, multi-formalism simulation.

The dynamic model of cortisol mediated signalling integrated with Recon 2 FBA model of human metabolism and PBPK models.
Multi-scale, multi-formalism simulation to integrate existing models.
Physiologically Based Pharmacokinetic Models (PBPK)

PBPK model of drug A and its toxic liver metabolite Atox detoxified through GSH conjugation (e.g. paracetamol and NAPQI).

Model structure and parameters adapted from Jones and Rowland Yeo 2013 CPT: Pharmacometrics & Systems Pharmacology Volume 2, Issue 8, pages 1–12, August 2013.

Simulation in qsspn.

www.surrey.ac.uk
Integration of molecular network models with PBPK.

Expanding Pharmacogenomics by 2192 genes. Expanding DDI analysis by 7440 potential targets. Enabling incorporation of ~omics data.


www.surrey.ac.uk
MUFINS: Multi-Formalism Interaction Networks Simulator.

Snoopy: External Petri Net Editor

JyMet: Mufins Graphic User Interface

> spept2qsspn

> qsspn

> sfba

Command line tools

http://sysbio3.fhms.surrey.ac.uk/mufins/

Manuscript in third stage of review in NPJ: Systems Biology & Applications.
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