UK Quantitative Systems Pharmacology Network

Exchange Workshop 1

5th to 7th September 2016

University of Surrey, UK.

Welcome!

Welcome to the first Exchange Workshop of the EPSRC/MRC co-funded UK Network on Quantitative Systems Pharmacology (QSP).

This meeting brings together an exciting line-up of invited and contributed talks from speakers with expertise in multiscale modelling, disease progression, parameter estimation and identifiability, and translation. Some of these talks will provide QSP application examples, some will detail theory applications to related areas as examples of good practice to the QSP community, whilst others will discuss the fertile ground open to QSP researchers in helping to develop future pharmaco-therapeutics. Equally exciting is the sign-up of pharmacological, biology, life and physical scientists, in industry and academia, attending!

Whilst the week comprises talks and posters, time has been provided in the schedule for us all to sit and discuss the four themed areas, how QSP can benefit from research in related areas and how QSP can be effectively applied to problems in drug discovery and development. Please do get involved in the discussions, enjoy yourselves and most of all have fun!

We gratefully acknowledge funding from the Engineering and Physical Sciences Research Council (EPSRC) and the Medical Research Council and support from Pfizer, AstraZeneca and GlaxoSmithKline.

On behalf of the UK QSP Organising Committee we hope you enjoy this meeting and welcome your input and feedback.

Marcus Tindall (on behalf of the Organising Committee)

Organising Committee
Prof Leon Aarons (Manchester)
Dr Mike Chappell (Warwick)
Dr Lourdes Cucurull-Sanchez (GlaxoSmithKline)
Prof Gianne Derks (Surrey)
Dr Pinky Dua (Pfizer)
Dr Marcus Tindall (Reading)
Prof Ben Whalley (Reading)
Dr James Yates (AstraZeneca)

Meeting Venue
All activities will take place in the Rik Medik Building at the University of Surrey, Stag Hill Campus, Guildford, Surrey, GU2 7XH, United Kingdom. Lecture presentations will take place in 03 MS 01. Rooms 32 MS 01, 39 MS 02, 75 MS 02 and 80 MS 02 have been reserved for designated discussion sessions during the meeting.

Registration desk & name badge
This can be found in the main foyer of the Rik Medik Building. Your name badge serves as your unique identification for the meeting whilst on the Surrey University Campus. Please do remember to have it with you at all times so University staff and the workshop organisers know who you are and can give you access to the areas you require for the duration of the workshop.

Meeting Programme
A copy of this programme can be downloaded from
www.qsp-uk.net/surrey-2016.html.
Copies of the programme have also been provided on your memory stick and a few paper copies are available at the registration desk in the main foyer of the Rik Medik Building.

Morning/Afternoon tea and lunch
These will be served in the main foyer of the Rik Medik Building.

Accommodation
If you are a full delegate then an ensuite room has been reserved for you in University Court. You will be able to check in to your accommodation (at the reception desk) from 17.30 hours on Monday 5th September. Please remember to check out of your accommodation by 9am on the morning of Wednesday 7th September. All rooms are pre-paid and delegates, unless otherwise agreed with the conference organisers, will only need to pay for any extra costs on their departure.

Please be aware that the University has informed us that a team has been contracted to survey some University accommodation during the period of our workshop. Delegates should thus be aware that contractors may be present in the accommodation area during their stay.

Monday evening buffet meal
For full delegates this is included in your registration fee. Day delegates are welcome to join in the evening meal on Monday, but will need to pay for their meal (£15.95/person). Meals will be served in the Hillside Restaurant.

Reception and Workshop dinner
The Workshop Dinner will be held on the evening of Tuesday 6th September from 19.30 to 21.00 hours in the Green Room in Wates House. If you have indicated you wish to attend the dinner then this is included in your registration fee. The meal will be preceded by a short reception which will be held in the Wates House bar area, adjacent to the Green Room, starting at 18.45hrs. Complementary wine, soft drinks and juice will be available. If delegates wish to purchase other alcoholic drinks during the reception, then they will need to meet the cost of this.

Bar
A bar can be found in Wates House and is open until 10pm each night.

Wifi Access
Wifi access is via the Cloud service.

Parking permits
These can be obtained from the registration desk.

Posters
If you are displaying a poster please ensure this is placed on the poster display boards prior to the poster morning and after poster sessions on Tuesday 6th September.

Luggage store
A secure luggage store (room 81 MS 02 of the Rik Medik Building) will be available to all delegates on Wednesday 7th September. Please go to the registration desk to gain access.

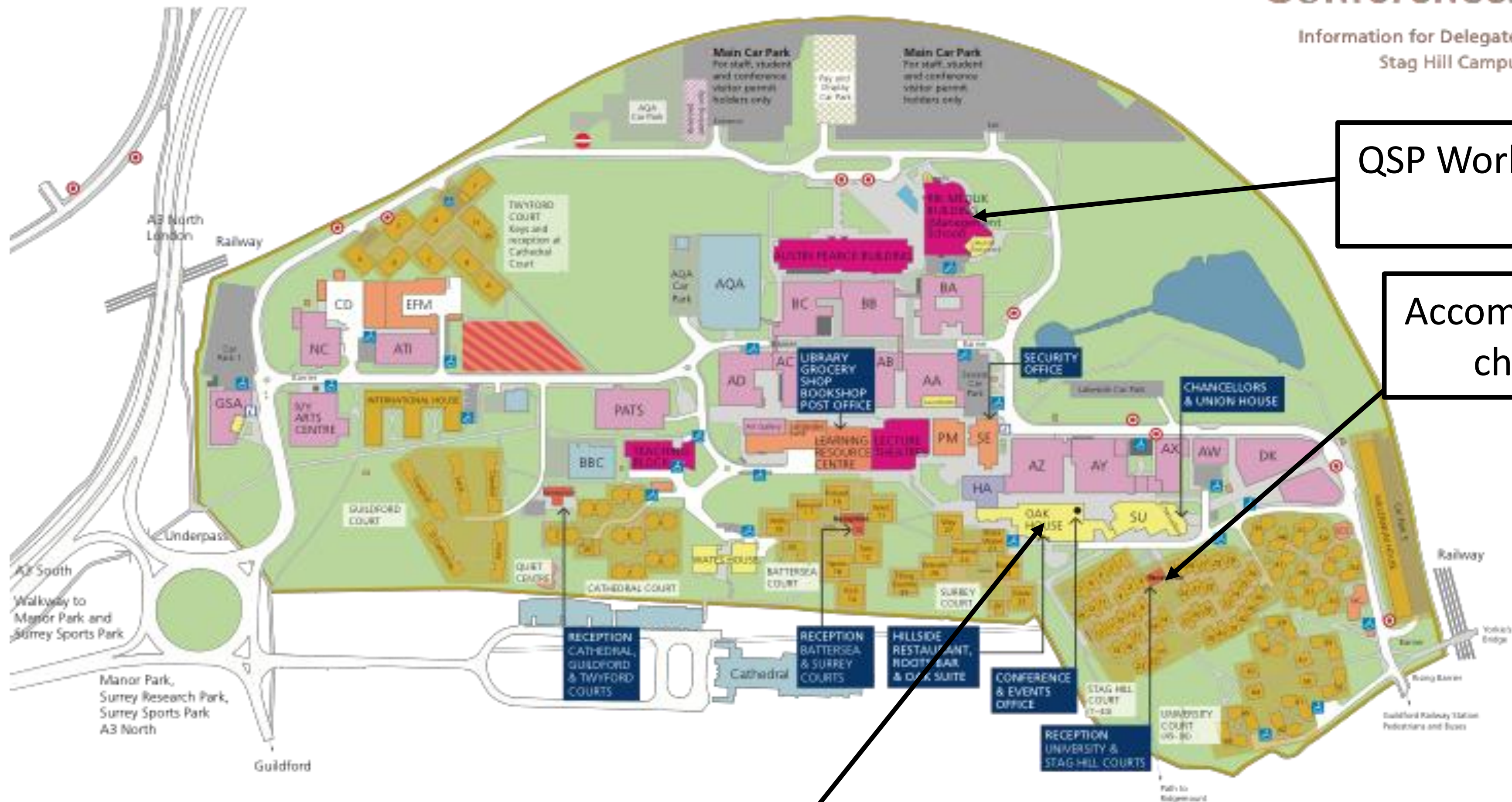
Delegate e-mail addresses
Delegate e-mails have not been included in this programme given it will appear on the Internet. A separate delegate e-mail list is available from the registration desk.

QSP website
Please see www.qsp-uk.net for all details on the UK QSP Network. If you have any suggestions or queries, please contact Marcus Tindall or a member of the Organising Committee.

Meeting queries
If you have any queries during the meeting please contact Tracey Simons (Meeting Administrator), Marcus Tindall or Gianne Derks in the first instance. We will do our utmost to accommodate any requests.

Surrey Conferences

Information for Delegates
Stag Hill Campus



www.surreyconferences.co.uk

QSP Workshop

Accommodation check-in

Evening meals

Programme

Monday 5th September

10.00-11.00 Registration & coffee – Foyer of Rik Medik Building
11.00-11.15 Welcome
Multiscale Modelling
11.15-12.00 ***A multi-scale systems pharmacology approach for anticancer chemotherapy personalisation*** Dr Annabelle Ballesta (University of Warwick)
12.00-12.45 ***Applying multi-scale models in immunotherapeutic discovery and development*** Dr Mark Coles (University of York)
12.45-13.45 Lunch – Foyer
13.45-14.05 ***Mathematical modelling of three dimensional cell culture and bioreactors for liver toxicity testing*** Dr Steven Webb (Liverpool John Moores University)
14.05-14.25 ***Dynamic, genome-scale modelling of the interplay between stress, circadian rhythms and metabolism*** Dr Nick Plant (University of Surrey)
14.25-14.45 ***Development of a dynamic multi-scale, computational model of human hepatic glucose and fructose metabolism*** Elaina Maldonado (University of Surrey)
14.45-16.00 Breakout group discussion
16.00-16.30 Afternoon tea – Foyer
16.30-17.15 Breakout group discussion continued
Disease Progression
17.15-18.00 ***The impact of modeling disease progression on refining clinical, drug development*** Dr Alex Phipps (Roche)
18.00-19.00 Accommodation check-in
19.00-20.30 Dinner

Tuesday 6th September

9.00-9.45 ***Epileptogenesis: the development and progression of epilepsy*** Prof Matthew Walker (University College London)
9.45-10.05 ***Mathematical model to study the interplay between tight junction and stratum corneum barriers for skin barrier homeostasis*** Mark van Logtestijn (Imperial College London)
10.05-10.25 ***Understanding Nrf2 – NF-κB crosstalk via mathematical modelling*** Darren Conway (Liverpool John Moore's University)
10.25-11.15 Morning tea with posters – Foyer
11.15-11.35 ***Development of a dynamic multi-scale, computational model of breast cancer***

A. Barber (University of Surrey)
Parameter estimation and identifiability
11.35-12.20 ***Computational models as microscopes: rate-limiting steps in proteasomes*** Dr Juliane Liepe (Imperial College London)
12.20-13.05 ***Structural Identifiability Analysis for QSP*** Dr Mike Chappell (University of Warwick)
13.05-14.00 Lunch – Foyer
14.00-15.30 Breakout group discussion
15.30-16.00 Sharing breakout group discussion
16.00-16.30 Afternoon tea with posters – Foyer
16.30-16.50 ***Modelling drug-target binding in a microfluidic flow tube containing cell culture*** Dr Vivi Rottschäfer (University of Leiden)
16.50-17.10 ***Dynamic modelling of oestradiol disposition and metabolism in humans: How increased parameterization can lead to increased biological insight*** Dr Nick Plant (University of Surrey)
17.10-17.30 ***Structural Identifiability of mixed-effects models: Methods and insights*** David Janzén (University of Warwick)
17.30-17.50 ***Input estimation for drug discovery using optimal control and Markov Chain Monte Carlo approaches*** Magnus Trägårdh (University of Warwick)
17.50-18.10 ***Third generation turnover model – Nicotinic acid-induced adaptation of insulin and free fatty acids*** Robert Andersson (University of Warwick)
18.10-18.45 Break
18.45-19.30 Reception
19.30-21.00 Workshop dinner

Wednesday 7th September

9.00-9.30 Network & funding update
Translation
9.30-10.15 ***QSP in Drug Discovery and Development - A Pillar for Translational Research*** Dr Pinky Dua (Pfizer)
10.15-11.00 ***TBA*** TBC
11.00-11.30 Morning tea – Foyer
11.30-11.50 ***Nonclinical to clinical translation of biomarkers for drug-induced effects on cardiac conduction*** Linnéa Bergenholm (University of Warwick)
11.50-12.10 ***Towards a 3D spatial distribution model of the brain*** Esmée Vendel (University of Leiden)
12.10-13.10 Lunch - Foyer

13.10-13.30	***Redundancy, Degeneracy, and Robustness in Protein-Interaction Networks*** Alice Schwarze (University of Oxford)
13.30-13.50	***Assessment of Mitochondrial Toxicity Using a pH-Dependent Model of Human Hepatic Cellular Bioenergetics*** Ross Kelly (Liverpool John Moores University)
13.50-14.10	***A systems pharmacology approach for prediction of pulmonary and systemic pharmacokinetics and receptor occupancy of inhaled drugs*** Elin Boger (University of Warwick)
14.10-15.00	***Systems Analysis of G protein-coupled Receptors Pharmacology*** Dr Graham Ladds (University of Cambridge)
15.00-15.30	Afternoon tea – Foyer
15.30-16.15	***Challenges of paediatric drug development: opportunies for QSP*** Dr Amy Cheung (AstraZeneca)
16.15	Close of meeting

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Talk Abstracts

Underlined names for contributed talks indicates the speaker

Theme-Multiscale Modelling

Invited talks

A multi-scale systems pharmacology approach for anticancer chemotherapy personalisation

Dr Annabelle Ballesta
Systems Biology Centre,
University of Warwick

Anticancer chemotherapy personalisation needs to reliably account for the activation of molecular pathways triggered by drug administration in each individual patient. Indeed, underlying gene and protein intracellular networks ultimately drive treatment antitumor efficacy and side effects in cancer and healthy tissues respectively, and they highly depend on patient-specific and tumour-specific genetic mutations or epigenetic alterations. However, clinical molecular data is usually minimally available in individual patients so that physiologically-based models designed through multi-scale approaches integrating preclinical and clinical investigations appears as an adapted solution. The models are based on ordinary differential equations (ODEs), and represent the relevant intracellular protein networks together with the pharmacokinetics-pharmacodynamics (PK-PD) of drugs of interest in the tumour and the healthy tissues for which chemotherapy is toxic. While PK quantifies the transport and metabolism of the drug and its metabolites that are driving exposure concentration over time, PD quantifies drug interactions with cellular targets and subsequent cytotoxicity.

Basing mathematical models on physiology allows the use of in vitro studies to design whole-body preclinical rodent models, to be further scaled to patient population data. Partial re-calibration of the resulting human model for a given cancer patient according to individual biomarker recordings, genetic background and therapeutic history further allow for chemotherapy personalisation. The patient-specific models would then initiate a novel kind of clinical trial in which each individual patient would receive personalised drug combinations/scheduling computed via mathematical models informed with a continuous flow of multidimensional information obtained and tele-transmitted from patients.

I will first present how this approach was undertaken for personalising cancer chronotherapeutics, that is administering anticancer drugs according to the patient's biological rhythms over the 24h span. The project focuses on the anticancer drug irinotecan, one of the three drugs of the current gold standard protocols against digestive cancers [1-3] . Then, I will present a multi-scale approach to design clinically-relevant models of the PK-PD of temozolomide, the cornerstone of treatments against brain tumours, and how they can be used to personalize combination chemotherapies [4].

References

1. Dulong, S., et al., *Identification of Circadian Determinants of Cancer Chronotherapy through In Vitro Chronopharmacology and Mathematical Modeling.* Mol Cancer Ther, 2015.
2. Ballesta, A., et al., *A systems biomedicine approach for chronotherapeutics optimization: focus on the anticancer drug irinotecan*, in *New Challenges for Cancer Systems Biomedicine*. 2012, Springer.
3. Ballesta, A., et al., *A combined experimental and mathematical approach for molecular-based optimization of irinotecan circadian delivery.* PLoS Comput Biol, 2011. **7**(9): p. e1002143.
4. Ballesta, A., et al., *Multiscale design of cell-type-specific pharmacokinetic/pharmacodynamic models for personalized medicine: application to temozolomide in brain tumors.* CPT Pharmacometrics Syst Pharmacol, 2014. **3**: p. e112.

Applying multi-scale models in immunotherapeutic discovery and development

Dr Mark Coles
Centre for Immunology and Infection,
University of York

Multi-scale computational models provide an emerging technology in the discovery and development of immuno-therapeutics and provide a link between 'omics' datasets and disease simulations. Inspired by critical systems engineering we have developed transparent evidence based models of autoimmune disease pathologies. Sjogren's syndrome is an exemplar autoimmune disease affecting approximately 1% of the population characterised by loss of secretions from the salivary and tear glands. This pathology is driven by antibody production in tertiary lymphoid tissue found in the salivary gland. We have developed a multi-scale hybridised agent based model incorporating cellular automata, generative grammar, ODEs, PDEs and Monte Carlo methods that replicates key aspects of salivary gland pathology during disease progression. Although animal models have been very useful in understanding mechanisms in the initiation of pathology they have been poor prognostic models of treating established pathology found in humans. By using a combination of high through-put model visualisation tools and Kohonen self-organising feature maps that allow exploration of virtual therapeutic interventions, we have identified a unique intervention strategy that has the potential to modulate established pathology. We are applying this approach to address both basic mechanisms of immune function, identify novel disease modulators and determine biomarkers of disease progression and therapeutic efficacy.

Contributed talks

Mathematical modelling of three dimensional cell culture and bioreactors for liver toxicity testing

S.D. Webb[1], S. McGinty[2], J. Firman[3], P. Sharma[3], M. Ellis[4], K. Leutchford[4], R. Shipley[5]
[1]Liverpool John Moores University, Liverpool, U.K.; [2]University of Glasgow, Glasgow, U.K.; [3]University of Liverpool, Liverpool, U.K.; [4]University of Bath, Bath, U.K.; [5]University College London, London, U.K.

Prediction of drug efficacy and human toxicity from animal or in vitro data continues to be one of the biggest challenges facing the pharmaceutical industries. For example, many in vitro assays are limited by their inability to mimic in vivo tissue architecture or reproduce responses due to drug exposure over extended time periods. The need for better predictive in vitro models for efficacy and toxicity is therefore clear. A clear lacking feature of most in vitro liver models is an inability to reproduce liver zonation. This reproduction of centrilobular and periportal hepatocyte phenotype in in vitro culture is crucial to their more sensitive detection of cellular stress. Hepatocyte metabolism and phenotype is dependent upon the cell position along the liver lobule, with differing exposure to substrate, oxygen and hormone gradients.
We are currently developing and comparing three new in vitro technologies with the aim of identifying optimal regimes for the recapitulation of in vivo liver zonation. The three technologies are: hepatospheres - 3-D microtissues aggregates; the Kirkstall Quasi-Vivo QV900 cell culture system - consisting of a series of connected sealed chambers within which cells can be cultured in a low shear stress flow environment; and, hollow fiber bioreactors - which are a 3D culture system that consist of fibers fixed into a module with cells seeded on the outside of the porous fibers and media delivered through the fiber lumen. The capacity to what degree the commercially available HEPG2/C3A human liver cell line can replicate in vivo responses is assessed in each technology.

The development of these systems is critically underpinned by mathematical and computational modelling to inform their operating set-ups, interpret data from system outputs and aid in optimizing design to mimic certain hepatic physiological conditions. Additionally, the mathematical modelling has been used to identify the key system parameters that will affect drug efficacy and clearance. The analysis of this combined in vitro and mathematical modelling work has produced novel results that allow the operating set-up to be calculated, and predictions of drug clearance to be generated efficiently.

Dynamic, genome-scale modelling of the interplay between stress, circadian rhythms and metabolism

Nilgun Sahin[1], Cheryl Isherwood[2], Jonathan Jonston[2], Debra J. Skene[2], Andrzej M. Kierzek[2], Hans V. Westerhoff[1,3, 4] and Nick Plant[2]
[1]Molecular Cell Physiology, Netherlands Institute of Systems Biology, VU University Amsterdam, de Boelelaan 1085, NL-1081 HV Amsterdam, The Netherlands. [2]School of Biosciences and Medicine, Faculty of Health and Medical Sciences, University of Surrey, Guildford, GU27XH, UK. [3]Manchester Centre for Integrative Systems Biology, Manchester Interdisciplinary Biocentre, University of Manchester, 131 Princess Street, Manchester M1 7DN, UK. [4]Netherlands Institute for Systems Biology, Swammerdam Institute for Life Sciences, the University of Amsterdam, EU.

The emergence of the twenty-four hour society has brought to prominence a number of unique health challenges. For example, shift work and transatlantic travel both result in desynchrony of the circadian clocks. In addition, constant demands on an individual's time can lead to increased stress episodes. In both cases, these effects have been associated with an increased incidence of metabolic syndrome, diabetes, cardiovascular disease and cancer. However, the mechanistic rationale for these associations remains elusive, limiting risk mitigation or management strategies. As the hormone cortisol plays a key role in both circadian timing/mechanisms/biology and stress responses, we have generated the first model to encompass circadian rhythms, nuclear receptor-dependent gene regulation, and genome-scale metabolism. We demonstrate the model can reproduce the known circadian rhythm of metabolic enzymes and transporters, both in terms of protein expression and reaction flux. In addition, predicted blood cortisol, glucose and amino acid levels closely match those measured in patient plasma, validating the models ability to reproduce known biology. Using this model, we demonstrate that increased plasma glucose is an emergent phenotype from either high frequency acute stress episodes or chronic circadian desynchrony. In addition, the combination of these two effects leads to an exacerbation glucose production consistent with an increased risk of metabolic syndrome. In conclusion, we demonstrate the utility of a multi-scale, multi-formalism modelling approach to understand the genotype-phenotype relationship underpinning a pathology of huge economic and healthcare significance.

Development of a dynamic multi-scale, computational model of human hepatic glucose and fructose metabolism

E.M. Maldonado[1], M.J. Tindall[2,3], N.J. Plant[1], A.M. Kierzek[1, 4] and J.B. Moore[5]
[1]School of Biosciences and Medicine, University of Surrey, UK. [2]Department of Mathematics & Statistics, University of Reading, UK. [3]Institute of Cardiovascular and Metabolic Research, University of Reading, UK. [4]Simcyp Limited (a Certara Company), Sheffield, UK. [5]School of Food Science and Nutrition, University of Leeds, UK.

Very high doses of fructose alter human hepatic insulin sensitivity and increase lipogenesis. However, the relevance of these data to population consumption is unclear. The aim of this work is to develop a multi-scale model of human hepatic monosaccharide transport, signalling and metabolism and predict the regulatory and metabolic outcomes to physiological levels of glucose and fructose in healthy and fatty liver.

Utilising quasi steady state Petri nets (QSSPN; Fisher, *et al.* 2013 *Bioinformatics*) together with our novel analysis approach 'dynamic flux variability analysis' (dFVA), the exchange flux of interest was set as the objective function. The minimal and maximal objective function values were used to calculate upper and lower bound time courses of metabolite concentration in the extracellular space. These bounds were consistent with stoichiometric and thermodynamic constraints of the model, whilst also satisfying the demands of a 'healthy hepatocyte' biomass function. Alongside this, an immortalised hepatocyte cell line, HepG2, was used to provide *in vitro* data to experimentally validate *in silico* predictions.

To date, we have reconstructed a dynamic regulatory network of hepatic glucose and fructose transport using the Petri net formalism and integrated this with HepatoNet1 constrained by *in vitro* flux data (Jain, *et al.* 2012 *Science*). QSSPN dFVA simulations have predicted minimum and maximum transport rates allowing the calculation of extracellular glucose and fructose concentrations

over time. HepG2 medium glucose and fructose concentrations were found to be within our predicted dFVA solution space. Preliminary simulations of a published kinetic model of hepatic insulin signalling (Kubota, *et al.* 2012 *Molecular Cell*) have been implemented in COPASI and successfully replicated by QSSPN.

In conclusion, we are able to reproduce hepatic monosaccharide uptake *in vitro* in our *in silico* model. Integration of a published model of the regulatory insulin signalling network allows prediction of the effects of insulin regulation on sugar and lipid metabolism in response to physiological levels of glucose and fructose in healthy and fatty liver.

Theme-Disease Progression

Invited talks

The impact of modeling disease progression on refining clinical, drug development

Dr Alex Phipps
Roche

Over the last few years mathematical models of disease progression for both chronic and acute conditions has led to key decisions being made on drug development programmes. Examples will be presented across Disease Areas where the ability to mathematically describe a biological condition and the effect of an intervention can be used to help objective decision making and to guide programme design.

When disease modeling is fully integrated into a drug programme or a disease area strategy if affords a more informed approach to deciding between many clinical permutations which can't easily be conceived in totality by a team. Challenges remain regarding the speed of implementation to make a timely intervention on a programme, the complexity of the work and the perceived 'fear factor' among a recipient non-mathematically trained project team and our ability to simplify communication and have maximum impact.

Epileptogenesis: the development and progression of epilepsy

Prof Matthew Walker
Institute of Neurology
University College London

Contributed talks

Mathematical model to study the interplay between tight junction and stratum corneum barriers for skin barrier homeostasis

Mark van Logtestijn[1], Georgios Stamatas[2], Reiko J. Tanaka[1]
[1] Department of Bioengineering, Imperial College London, London, UK. [2] Johnson and Johnson Santé Beauté, France.

The skin consists of two barriers, the stratum corneum (SC) and the tight junction (TJ) in the stratum granulosum (SG), which work together to maintain the skin's permeability barrier. The mechanisms responsible for skin barrier homeostasis are yet to be elucidated, mainly due to a current lack of experimental tools to evaluate the TJ barrier function *in vivo*.

To study the regulatory mechanisms responsible for the interplay between the TJ and SC barriers to achieve the barrier homeostasis, we developed a partial differential equation model that describes spatio-temporal regulation of the SC barrier components (corneodesmosomes, intercellular lipids, natural moisturizing factor) via modulation of Ca^{2+} concentrations and pH in the SC/SG

interface. The model is a natural extension of the recently proposed characterisation of the depth-dependent SC resistance (van Logtestijn et al. 2015, PLoS ONE).

The simulation results will be compared and calibrated with the experimental data on SC barrier damage and suggest how the impaired TJ barrier causes SC barrier defects via perturbed regulation of pH and Ca^{2+} concentrations. The obtained results on the skin's permeability barrier and its depth-dependency can refine models on dermal drug absorption and permeation for healthy and defective skin.

Understanding Nrf2 – NF-κB crosstalk via mathematical modelling

Darren Conway[1], Joe Leedale[2], Joanna Wardyn[3], Chris Sanderson[3], Steve Webb[1]
[1]Department of Applied Mathematics, Liverpool John Moore's University, James Parsons Building Byrom Street, Liverpool, L3 3AF, UK. [2]Department of Molecular and Clinical Pharmacology, University of Liverpool Sherrington Building, Ashton St., Liverpool, L69 3GE, UK. [3]Department of Cellular and Molecular Physiology, University of Liverpool, Crown Street, Liverpool L69 3BX, UK

Reactive oxygen species (ROS), produced by oxidative stress, are a by-product of normal metabolism and play a role in the initiation, promotion, and malignant conversion of carcinogenesis through activation/suppression of redox-sensitive transcription factors. NF-E2-related factor 2 (Nrf2) encodes for antioxidant and general cytoprotective genes, while NF-κB regulates the expression of pro-inflammatory genes. During low levels of oxidative stress, Nrf2 is activated and translocates to the nucleus where it transcribes its target genes preventing a further increase in ROS levels restoring oxidative modifications of affected proteins. However, at a certain threshold at which ROS levels threaten cellular viability, the NF-κB responses become activated, triggering an inflammatory response. The NF-κB and Nrf2 pathways are at the core of protective cellular signalling and the imbalance between their activities has been linked to many inflammatory diseases, cancer and neurodegeneration. The central goal of the proposed research is to develop an integrated *in vitro*/*in silico* model that will provide a better understanding of how the two pathways interact. A stochastic petri net model containing both Nrf2 and NF- κB pathways was generated and the correlation between this computational data and the experimental data provides confidence that the model predicts critical test points of contact between the two pathways. The model can then be used to advance the discovery, development and clinical use of therapeutic drugs that will target oxidative stress in cases of chronic inflammation and disease.

Development of a dynamic multi-scale, computational model of breast cancer

Amy Barber[1], M. Ajaz[1], A.M. Kierzek[1, 2] and N.J. Plant[1]
[1]School of Biosciences and Medicine, University of Surrey, [2]Simcyp Limited (a Certara Company), Sheffield

The multifactorial nature of breast cancer is a major barrier to the development of effective treatment. Despite recent and on-going advancements, breast cancer remains the most common cause of cancer deaths globally. Inter- and intra-tumoral heterogeneity contributes to differences in aggressiveness, prognosis and therapeutic response, complicating treatment. In particular, the development of targeted therapeutics against triple-negative breast cancers has had limited success. This provides motivation for the development of novel methodologies for breast cancer drug discovery. We show that through the use of mathematical modelling and flux distribution analysis of genome scale metabolic networks

Here we show the development of a dynamic model integrating genome-scale metabolism, a gene and signalling regulatory network centred on the estrogen and progesterone receptors, and a kinetic model of the cell cycle. We have constrained both parts of the network using microarray data, producing reconstructions for several breast cancer cell lines. In addition, we further constrain the model using in vitro metabolomics flux data. We demonstrate that this model can reproduce behaviours of in vitro cell lines: consumption of glucose and lactate; response to metabolic drugs; response to cancer chemotherapeutics.

The cell- and tumour- specific models provide novel opportunities for in silico testing of cancer cell responsiveness to different experimental conditions, in particular identifying metabolic vulnerabilities as potential drug targets. We anticipate this will be invaluable to researchers studying the network biology of breast cancer. Furthermore, utilisation of this model will aid development of effective combinatorial treatments and begin to remove barriers to the reduction of mortality from breast cancer.

Theme-Parameter estimation and identifiability

Invited talks

Computational models as microscopes: rate-limiting steps in proteasomes

Dr Juliane Liepe
Theoretical Systems Biology Group
Imperial College, London

Proteasomal protein degradation is a key determinant of protein half-life and hence of cellular processes ranging from basic metabolism to a host of immunological processes. Despite its importance the mechanisms regulating proteasome activity are only incompletely understood. Here we use an iterative and tightly integrated experimental and modelling approach to develop, explore and validate mechanistic models of proteasomal peptide-hydrolysis dynamics. The 20S proteasome is a dynamic enzyme and its activity varies over time because of interactions between substrates and products and the proteolytic and regulatory sites; the locations of these sites and the interactions between them are predicted by the model, and experimentally supported. The analysis suggests that the rate-limiting step of hydrolysis is the transport of the substrates into the proteasome. The transport efficiency varies between human standard- and immuno-proteasomes thereby impinging upon total degradation rate and substrate cleavage-site usage.

Structural Identifiability Analysis for QSP

Dr Mike Chappell
School of Engineering, University of Warwick

For many systems (certainly those in pharmacology, biology and medicine) the mathematical models that are generated invariably include state variables that cannot be directly measured and associated model parameters, many of which may be unknown and which also cannot be measured. For such systems there is often limited access for inputs or perturbations. These limitations cause immense problems when investigating the existence of hidden pathways or mechanisms, or when attempting to estimate unknown parameters and this can severely hinder model validation. It is therefore highly desirable to have a formal approach to determine what additional inputs and/or measurements are necessary in order to reduce, or remove these limitations and permit the derivation of models that can be used for practical purposes with greater confidence.

Structural identifiability arises in the inverse problem of inferring from the known, or assumed, properties of a biomedical or biological system a suitable model structure and estimates for the corresponding rate constants and other parameters. Structural identifiability analysis considers the uniqueness of the unknown model parameters from the input-output structure corresponding to proposed experiments to collect data for parameter estimation (under an assumption of the availability of perfect, noise-free observations). This is an important, but often overlooked, theoretical prerequisite to experiment design, system identification and parameter estimation, since estimates for unidentifiable parameters are effectively meaningless. If parameter estimates are to be used to inform about intervention or inhibition strategies, or other critical decisions, then it is essential that the parameters be uniquely identifiable.

Numerous techniques for performing a structural identifiability analysis on linear parametric models exist and this is a well-understood topic. In comparison, there are relatively few techniques

available for nonlinear systems (the Taylor series approach, similarity transformation based approaches, differential algebra techniques and the more recent observable normal form approach) and significant (symbolic) computational problems can arise, even for relatively simple models.
Structural indistinguishability for systems models is concerned with determining the uniqueness between possible candidates for the model (or mechanism) structure. The analysis is concerned with whether the underlying possibilities for the parameterised mathematical model can be distinguished using the inputs (perturbations or interventions) and observations (or measurements) available for the system under investigation.

For linear PKPD systems the analysis is generally exhaustive with all competing mechanisms generated from a given one , but for nonlinear systems the approach is generally only for pairs of candidate models, though in some cases a parameterised family of such candidates can be generated.
In this talk an introduction to structural identifiability and indistinguishability analyses will be provided demonstrating the application of the techniques available to both linear and nonlinear systems to examples of Systems Pharmacology models of various degrees of complexity.

Contributed talks

Modelling drug-target binding in a microfluidic flow tube containing cell culture

Vivi Rottschäfer[1], Wilbert deWitte[2], Elisabeth C.M. de Lange[2].
[1]Mathematical Institute, Leiden University, P.O. Box 9512, 2300 RA Leiden, the Netherlands.
[2]Division of Pharmacology, Leiden Academic Centre for Drug Research, Leiden University, Leiden, The Netherlands.

Drugs act in the body by binding to receptors which results in an effect. The rates with which the drugs, also called ligand, bind to receptors and with which they detach from the receptors are in general unknown. Moreover, it is very difficult – sometimes even not possible – to measure these binding rates directly in experiments. Our aim is to estimate the binding rates by combining in vitro experiments with a mathematical model.

In the experiments, we use a flow channel containing cells with receptors to which the ligand can bind. We produce a flow through this channel of a fluid containing a concentration of ligand. Then, the concentration of the ligand at the end of channel resulting from the input-flow combined with the binding to receptors is measured.

Mathematically, we model this set-up by a system of differential equations for the ligand and the bound ligand-receptor complex. The ligand moves through the channel by advection (flow) and diffusion while simultaneously binding to receptors. The receptors, and hence, also the bound complex is fixed in space.

These result in an advection-diffusion equation (a partial differential equation (PDE)) for the ligand coupled to an ordinary differential equation (ODE) for the bound complex. We study this system of differential equations by numerical simulations and analytical methods.

By combining the results obtained with the mathematical model and the results from the experiments we estimate the order of magnutide of the rates of the binding kinetics.

Dynamic modelling of oestradiol disposition and metabolism in humans: How increased parameterization can lead to increased biological insight

Joanna H. Sier, Alfred E. Thumser and Nick J. Plant
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Oestrogen is a vital hormone that regulates a large number of biological functions within the body. These include roles in the development of the secondary sexual organs in both sexes, plus uterine angiogenesis and proliferation during the menstrual cycle and pregnancy in women. Deregulation of oestrogen biology has been associated with a number of adverse effects, most notably carcinogenesis. Oestrogen biology may be altered deliberately through oestrogen-containing conception and hormone-replacement therapy, or accidently through environmental exposure to oestrogen-like endocrine disruptors. It is thus important to build predictive models to examine risk assessment scenarios. We present a traditional physiologically based pharmacokinetic model of oestrogen,

demonstrating that it is able to reproduce the observed plasma kinetics in humans following intravenous bolus and infusion exposure of oestrogen. Next, we reconstruct a detailed kinetic model of oestrogen metabolism in the liver, and demonstrate it can reproduce the metabolism of oestradiol in primary human hepatocytes. Finally, we integrate these two models to generate a hybrid PBPK model where the intrinsic clearance expression is replaced by a fully parameterized deterministic model. This hybrid model is again able to reproduce the human in vivo pharmacokinetic data, but with an added level of data richness. To demonstrate the advantage of this higher level of mechanistic information, we examine the role of COMT genotype in determining blood concentration of the mutagenic catecholoestrogens in a virtual patient population. While hepatic COMT activity is reduced to one-third wild-type, no increase in circulating catecholoestrogens is predicted, providing mechanistic support for the epidemiological data. In summary, we demonstrate (i) the utility of integrating models to generate larger reconstructions, and (ii) the power of such an approach to generate significant biological insight into genotype-phenotype interactions.

Structural Identifiability of mixed-effects models: Methods and insights

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Structural identifiability is a prerequisite to successful parameter estimation. If a model is structurally globally identifiable there exists a unique solution to the inverse problem. This is of particular importance in modelling of biological systems where conclusions are often drawn from the parameter estimates following the parameter estimation.

In this work the concept of structural identifiability has been generalised in the sense that it now also includes the commonly used mixed-effects modelling framework. Several analytical methods applicable to study structural identifiability of mixed-effects models have been developed. These methods can handle both linear and nonlinear structural models, both diagonal and non-diagonal covariance matrices and arbitrary distribution of the random effects, e.g., normal/lognormal. The developed methods can be used to determine whether the distribution of the set of output functions uniquely, or otherwise, determines both the structural parameters as well as the statistical parameters.

Interesting results has followed from the application of the developed techniques on mixed-effects models. It turns out that results from structural identifiability analysis of non-mixed-effects models does not necessarily hold for the corresponding mixed-effects model. In other words, a model may go from being unidentifiable/locally identifiable in a non-mixed-effects setting to become locally/globally identifiable in a mixed-effects settings. We have shown that this is related to the random effects in three different ways *i)* Where the random effects enter into the structural model *ii)* The form of the random effects *iii)* The structure of the covariance matrix related to the random effects.

To exemplify this, mixed-effects versions of a locally identifiable three-compartment model and the well-known unidentifiable one-compartment absorption model are analysed with the developed methods. It is shown, with a particular choice of random effects, that the models become globally identifiable and (at least) locally identifiable respectively.

Input estimation for drug discovery using optimal control and Markov Chain Monte Carlo approaches

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Background: Input estimation is employed when analysing dynamical systems in cases

where it is desirable to recover the form of an input function which cannot be directly observed and for which there is no model for the generating process. In pharmacokinetic (PK) and pharmacodynamic (PD) modelling, input-estimation in linear systems (deconvolution) is well established. However, many PKPD models are nonlinear, e.g. as a result of saturable elimination. Therefore, it is important to be able to handle models with nonlinearities. Input estimation in PKPD applications is challenging since sampling is sparse compared to typical engineering applications.

Aim: The aim of this work is to provide a rigorous definition of the input-estimation problem, and to discuss and illustrate the choices involved in terms of modelling assumptions and estimation algorithms using realistic case studies.

Methods: We cover *Maximum a Posteriori* estimates using various techniques from the field of optimal control, and full Bayesian estimation using Markov Chain Monte Carlo (MCMC) approaches. Traditional MCMC methods as well as the modern Riemannian manifold sampling methods are investigated. These techniques are implemented using the optimisation software CasADi, and applied to two example problems: one where the oral absorption rate and bioavailability for the drug eflornithine are estimated using PK data from rats, and one where energy intake is estimated from body-mass measurements of mice. The results from the analysis are used to highlight the strengths and weaknesses of the methods when applied to sparsely sampled data from drug-discovery projects.

Results: The presented methods for optimal control are fast and robust, and can be recommended for use in drug-development projects. The MCMC-based methods can have long running times and require more expertise from the user. However, it is possible in certain cases to handle even high-dimensional estimation problems efficiently with little or no manual tuning.

Conclusion: The rigorous definition together with the illustrative examples and suggestions for software tools serve as an excellent starting point for application of input-estimation methods to problems in drug discovery.

Acknowledgements: This work is funded through the Marie Curie FP7 People ITN European Industrial Doctorate (EID) project, IMPACT (Innovative Modelling for Pharmacological Advances through Collaborative Training). Project Number: 316736.

Third generation turnover model – Nicotinic acid-induced adaptation of insulin and free fatty acids

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Nicotinic acid (NiAc) is a potent inhibitor of adipose tissue lipolysis and acute administration results in a rapid reduction of plasma free fatty acid (FFA) concentrations. However, sustained NiAc exposure is associated with tolerance development, with FFA returning to pretreatment levels (complete adaptation). Furthermore, a major FFA rebound is seen upon abrupt NiAc washout. Although previous mathematical models successfully describe the acute NiAc-FFA concentration-response relationship, complete adaptation following long-term NiAc exposures requires a new model. In the present study, a 3rd generation turnover model has been developed that describes the adaptation in plasma FFA concentrations following long-term NiAc exposures in lean and obese rats. This was accomplished by extensively modifying previous NiAc-FFA models, resulting in a more general model which is able to capture FFA responses following both acute and repeated NiAc exposures. Specifically, insulin was incorporated alongside NiAc as a co-driver of FFA dynamics. Moreover, the drug-induced efficacy in the system was modelled as a flexible mechanistic function which allowed the system to attain complete adaptation upon sustained NiAc exposure. The pharmacokinetic/pharmacodynamic (PK/PD) models were challenged with an extensive pre-clinical data set comprising a variety of different NiAc provocations. NiAc infusions ranged from 1 h to 5 days, including continuous (24 h/day) and intermittent (12 h/day) protocols, achieved using

implantable, programmable mini-pumps. In addition, an engineered gradual NiAc withdrawal protocol was explored as a means of attenuating FFA rebound development. Remarkably, FFA rebound was actually increased during gradual NiAc withdrawal, likely due to NiAc-induced inhibition of insulin secretion; highlighting the importance of including endogenous agonists when modelling metabolic systems. In conclusion, the structure of the 3rd generation turnover model adequately captures a wide range NiAc-induced FFA responses, including acute, intermittent and sustained exposures, and may become a powerful predictive tool to further rationalize NiAc-induced antilipolytic dosing regimens.

Theme-Translation

Invited talks

QSP in Drug Discovery and Development - A Pillar for Translational Research

Dr Pinky Dua
Pfizer

Contributed talks

Nonclinical to clinical translation of biomarkers for drug-induced effects on cardiac conduction

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Objectives: To quantify the nonclinical to clinical translation of four anti-arrhythmics on QRS and PR intervals.

Introduction: Cardiac conduction liabilities are assessed in the clinic primarily through monitoring the QRS and PR intervals in the electrocardiogram (ECG). Nonclinical assessment include *in vitro* ion channel assays (e.g. Nav1.5, Cav1.2) and *in vivo* telemetry studies in e.g. guinea pig and dog. However, knowledge of the quantitative translations between nonclinical and clinical effects is limited.

Methods: Population PKPD models of QRS and PR intervals were developed for four anti-arrhythmics (AZD1305, flecainide, quinidine and verapamil) in guinea pigs (flecainide and verapamil), dogs [1] and humans. Top-down translational relationships between species were investigated by simulating the final models at matched exposures and the results visualised. In addition, the signal transduction between ion channel effects and QRS and PR prolongations in humans were investigated by modelling the clinical AZD1305 and *in vitro* data combined applying operational models.

Results: Top-down translations suggested that guinea pigs and dogs are less sensitive to drug-induced QRS and PR prolongations compared to humans. 10% QRS widening in humans compared to average prolongations of 2-3% in dogs (3 compounds) and 4% in guinea pigs (1 compound). 10% PR prolongation in humans compared to average prolongations of 3-10% in dogs (3 compounds) and 2-4% in guinea pigs (2 compounds). Middle-out modelling of QRS and PR prolongations induced by AZD1305 suggested that 10% QRS widenings correspond to 5-7% hNav1.5 inhibition while 10% PR prolongations correspond to 12-22% rCav1.2 DIL binding.

Conclusions: Quantitative translation methods of four anti-arrhythmics suggest that hNav1.5 inhibition and rCav1.2 binding of a few percent may produce clinically significant QRS and PR prolongations in humans. Also, dogs were up to 5 and 3 times less sensitive to QRS and PR prolongations respectively compared to humans, all with clear implications for cardiac safety assessment.

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Towards a 3D spatial distribution model of the brain

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To develop drugs that target the brain, a better understanding of the spatial behaviour of drugs in the brain in relation to binding to local targets is needed. This improves the prediction of their effects. To that end, it is important to realize that the brain is not a homogeneous tissue. There are many structures and differences between local tissue characteristics such as cerebral blood flow, brain cell types, target expression and brain fluid flow dynamics. Moreover, local tissue characteristics may be influenced by local brain diseases (e.g. a brain tumor). Currently, existing models for prediction of drug distribution and effect divide the brain into several physiological compartments. These include the brain extracellular fluid (brain ECF), brain tissue and several cerebrospinal fluid compartments, where characteristics in each physiological compartment are considered to be homogeneous.

On the way towards a 3D spatial drug distribution model of the brain, we first developed a 2D model that gives more detailed insight into the change of drug concentration in the brain both in time and space. It consists of several components: the blood, the blood-brain barrier, the brain ECF, the brain cells with their receptors and the cerebrospinal fluid. The drug is transported through the blood by diffusion and flow and enters the brain ECF through the blood-brain barrier. There, the drug is transported by an unidirectional bulk flow through the brain ECF of the units. Each unit consists of the cells and brain ECF that lie between two blood vessels and its size is based on physiological parameters. The drug may bind to receptors that are located on the surface of the brain cells. The model is represented by a system of partial differential equations that describe the concentrations of free drug in the brain ECF and the concentrations of free and bound receptors on the surface of the brain cells. Hereby, drug transport by diffusion and flow as well as receptor binding kinetics are taken into account. We study the system with analytical methods and numerical simulations.

Redundancy, Degeneracy, and Robustness in Protein-Interaction Networks

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Researchers in "network pharmacology" model complex biological systems as networks and use tools from network analysis to gain insights into biological problems. This approach can facilitate multi-target drug discovery. For example, one can associate biological systems with protein-interaction networks (PINs) and chemical compounds affecting these systems with network perturbations. By analysing structural changes induced by a perturbation on a PIN, it is possible to elucidate the systemic impact of a chemical compound on a biological system.

It is unclear how biological function relates to structural properties of the corresponding PINs. A better understanding of this relationship is needed to de_ne suitable measures for perturbations that network pharmacologists use to model the impact of a drug on a biological system.

In collaboration with e-Therapeutics, we aim to develop computational methods for identifying structural properties that may be linked to the integrity of biological functions in a cell. To that end, we identify links between biological and structural robustness of PINs and combine insights from evolutionary biology on the robustness of complex biological systems with _ndings on the percolation properties of PINs and random-graph models thereof.

The relationship between structure and function of biological networks has been of considerable interest to researchers in neuroscience. For PINs, we adapt a method that Tononi et al. [1] proposed for linking the structure of cortical networks to biological characteristics: We use information-theoretic measures for quantifying biological redundancy and degeneracy - two concepts that evolutionary biologists consider to be closely linked to robustness of biological systems - and link these quantities to small-scale structures (graphlets) in PINs.

References
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Assessment of Mitochondrial Toxicity Using a pH-Dependent Model of Human Hepatic Cellular Bioenergetics

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Drug induced mitochondrial dysfunction is an understated mechanism of toxicity responsible for the withdrawal of several drugs from the market. Recently, off-target toxicities of this nature have become more widely acknowledged, with more effort being assigned to better understanding the underlying mechanisms of mitochondrial toxicity. Extracellular flux analysis (Seahorse) is the current *in vitro* tool of choice for cellular bioenergetic toxicity assessment, allowing simultaneous measurement of mitochondrial respiration and glycolytic flux by monitoring cellular oxygen consumption rate (OCR) and extracellular acidification rates (ECAR) respectively.
Glucose metabolism (glycolytic flux) accounts for a significant portion of cellular bioenergetics as a direct source of ATP, and as a molecular precursor source for other energy producing systems such as the mitochondria via the TCA cycle. As a result, perturbations of cellular respiration, often as a result of "off-target" drug induced mitochondrial toxicity, may be observed by compensatory effects on glycolytic flux. As a biological system of high importance, particularly for drug toxicity, *in silico* models of hepatic glucose metabolism already exist. However, a comprehensive pH-dependent model that includes dynamic proton buffering and metal cation binding within hepatic cellular respiration and glucose metabolism has yet to be presented.

An *in silico* model of human hepatic bioenergetics, incorporating pH-dependent enzyme kinetics and reaction equilibria to compute the time course of pH changes has been constructed, showing that lactic acid transport into the extracellular environment is responsible for the pH changes as a result of increased glycolytic flux. The model has been coupled with *in vitro* ECAR and OCR data from extracellular bioenergetic flux analysis. Furthermore, coupling detailed thermodynamically driven, charge and mass balanced mathematical models with *in vitro* extracellular flux analysis has the potential to yield mechanistic insight into "off-target" toxicities such as drug induced mitochondrial dysfunction.

A systems pharmacology approach for prediction of pulmonary and systemic pharmacokinetics and receptor occupancy of inhaled drugs

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Background: Inhalation is an attractive route of administration for lung targeted drug treatment. The process of pulmonary drug disposition after inhalation is complex as it involves several mechanisms including particle deposition, particle dissolution and mucociliary clearance. Besides these complexities, there are no established methodologies to assess the lung target site concentration that exerts the pharmacological effect. As opposed to systemically acting drugs, the unbound concentration of drug in plasma cannot be expected to drive the target engagement for locally acting

inhaled drugs. An integrated understanding of the system as well as new experimental techniques are therefore desired.

Aim: To develop a systems pharmacology approach to mechanistically describe lung disposition in rats and compare predictions with novel generated experimental data of drug concentrations and receptor occupancy.

Methods: A mechanistic whole-body physiologically-based pharmacokinetic (PBPK) model with lung disposition was implemented in MATLAB. Due to the heterogeneous nature of the organ, the lung was divided into a central and a peripheral region. Fundamental processes were described by mathematical models, such as: 1) regional lung deposition, 2) particle dissolution, and 3) mucociliary clearance. Perfusion-rate limited kinetics was assumed to apply for all tissues.

By using the developed model and input parameters of a poorly soluble drug (fluticasone propionate, FP), *de novo* predictions of the PK were made after intravenous (IV) administration and nose-only inhalation. Except from the solubility, no parameters were estimated in the PBPK model.

Results: When drug- and formulation-specific input parameters of FP were fed into the developed model, it proved predictive of the PK and receptor occupancy after both IV-administration and nose-only inhalation, thereby allowing identification of key determinants of pulmonary selectivity of inhaled receptor occupancy: slow particle dissolution and slow drug-receptor dissociation.

Conclusion: The model is unique in its ability to distinguish between drug-, formulation- and system specific-properties. This enables assessment of important factors for lung targeting including properties of the molecule, formulation, the physiology of the animal species as well as the inhalation manoeuvre (nasal/oral), thereby providing a framework for rational drug design and translation of lung targeting from animal to human.

Acknowledgements: This work is funded through the Marie Curie FP7 People ITN European Industrial Doctorate (EID) project No.316736, IMPACT (Innovative Modelling for Pharmacological Advances through Collaborative Training).

QSP Excellence

Systems Analysis of G protein-coupled Receptors Pharmacology

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An essential prerequisite for cells is their ability to perceive and respond correctly to their microenvironment. Cells receive information from their neighbours, and their surroundings that informs their development, differentiation and eventually death. Errors or malfunctions in cellular signalling are responsible for diseases such as cancer, and diabetes. Many of these external signals are detected by cell surface G protein-coupled receptor (GPCR) signalling pathways. Understanding these important molecules, and their associated signalling pathways, is not purely an academic exercise since many pharmaceutical drugs, either directly or indirectly, target GPCRs. Pharmaceutical companies face growing challenges in bring new drugs to the market and it is apparent that new approaches to drug development are required. Our research is to aimed at develop new and novel techniques to address the challenges faced in predicting GPCR signalling behaviour. Through the combination of computational modellers, molecular pharmacologists and molecular biologists we are developing a quantitative systems approach to investigate GPCR pharmacology specifically aimed at understanding ligand-receptor-G protein selectivity. These concepts and approaches will be presented coupled to our latest projects looking at modelling GPCR signalling bias.

Prelude to UK QSP September 2017 Problem Workshop

Challenges of paediatric drug development: opportunies for QSP

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*Joint work with James W. T. Yates, Alex MacDonald, Diansong Zhou and Gabriel Helmlinger

Medicinal product development in neonates, infants and children is often challenging in terms of the understanding of rapid growth and development of the body, which can impact the observed pharmacokinetics (PK) and pharmacodynamics (PD) of drugs used, and the similarities and differences in disease pathophysiology and progression relative to adults. In recent years, focus and initiatives to improve drug discovery and development in children have been encouraged by regulators (*e.g*., EMA and FDA), professional groups (*e.g*., EFPIA) and paediatric networks (*e.g*., GRIP). These include the use of modelling methodology such as extrapolation between populations, Bayesian approaches, mechanistic models (*e.g*., PBPK) and frameworks (*e.g*., MID3 approach) to utilise existing data and knowledge in paediatric and adult trials. Much of the emphasis has been on the understanding of PK, hence understanding of how maturity/ontogeny alters system parameters, the levels of variability and uncertainty changes in critical biomarkers and their relationship to clinical outcome are still limited. To truly establish model continuum and the goals of QSP, a mechanistic understanding of the interaction of drugs with a complex, changing physiology and the resulting patient response is required. In this talk, we will outline some opportunities for QSP in paediatric drug development.

References

MID3 WG: White Paper Good Practices in Model-Informed Drug Discovery and Development (MID3): Practice, Application and Documentation.

Quantitative and Systems Pharmacology in the Post-genomic Era: New Approaches to Discovering Drugs and Understanding Therapeutic Mechanisms. An NIH White Paper by the QSP Workshop Group – October, 2011

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Poster Abstracts

Underlined names indicate those delegates exhibiting the poster.

Modelling and simulation of biased agonism dynamics at a G protein-coupled receptor

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G protein-coupled receptors (GPCRs) form the largest protein family in the human genome. Around 30% of marketed drugs target these receptors and therefore understanding their signalling pathways is not simply an academic exercise. For many years it had been incorrectly assumed that GPCRs bound a single agonist to elicit a response. However, there is now overwhelming evidence to suggest that many GPCRs can exist in multiple receptor conformations and can elicit numerous functional responses, both G protein- and non-G protein dependent. Furthermore, ligands have the potential to activate the different signalling pathways to varying extents - a concept referred to as biased agonism. The therapeutic promise of biased agonists is obvious; we could design ligands that actively engage with one beneficial signalling outcome while reducing the contribution observed at those that mediate more undesirable effects. Indeed, biased agonism probably explains why some drugs display clinical efficacy, while others fail, despite showing similar profiles of efficacy in preclinical trials.

Despite numerous ligands displaying biased agonism at particular receptors we do not, as yet, have a clear mechanistic understanding of the temporal dynamics exhibited by these compounds. Our approach to address this issue is to combine mathematical modelling and molecular pharmacology with a view to providing a systems-level understanding of biased agonism. Given that recent experimental results indicate a clear possibility for time dependent bias, whereby an agonist's bias with respect to different pathways may vary dynamically (Herenbrink et al, Nature Comms 2016), efforts towards understanding the implications of temporal bias by characterising and quantifying ligand effects on multiple pathways will clearly be aided by extending current equilibrium binding and biased activation models to include G protein-activation dynamics.

Here, we present a new model of time-dependent biased agonism, based on ordinary differential equations for multiple cubic ternary activation models with G protein cycle dynamics. The model is generally applicable to systems with n G proteins and N active receptor states. Numerical simulations for $n=N=2$ reveal new insights into the effects of system parameters (including cooperativities, and ligand and receptor concentrations) on bias dynamics. Further, we have 'fitted' this model to 'wet' experimental data for 2 competing G proteins (Galphai and Galphas) that become activated upon stimulation of the adenosine A1 receptor with adenosine and NECA (an adenosine derivative). We also show that our model can also qualitatively describe the temporal dynamics of this competing G protein activation. We believe that the data we describe here validates our use of this model for describing temporal G protein bias. We now aim to extend the use of this model to investigate the dynamics associated with therapeutically important glucagon-like peptide 1 receptor, a gut released hormone known for its insulinotropic effects.

Use of Mechanistic Models to Determine Kinetics Parameters of a Fluorescent Probe in the Presence and Absence of a Prototypical Inhibitor in HEK-OATP1B1 cells

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A recent paper by Izumi et al (2016) [1] proposed 2',7'-dichlorofluorescein (DCF) as a selective OATP1B1 probe substrate providing an alternative to [^{3}H]estradiol-17β-glucuronide for use during inhibition studies. This poster looks at the use of DCF alone (0.3-100μM) and its inhibition by gemfibrozil (an OATP1B1 inhibitor) across a range of concentrations (1-300μM) to investigate the mode of inhibition of gemfibrozil co-incubation with 1μM DCF in HEK-OATP1B1 cells using mechanistic models. The inhibition of the uptake of DCF by gemfibrozil was considered with three different scenarios: Michaelis-Menten (M-M) type competitive inhibition, Hill function type competitive inhibition and non-competitive inhibition (model 1-3 respectively). All three models

converged successfully within the Monolix Suite (Lixoft, France) and the M-M parameter values estimated were then compared to those obtained by [1]. A mechanistic model for DCF alone was also evaluated to gain understanding of the models that could be used in combination with gemfibrozil. A two compartment model consisting of media and cell with passive rate constants and a saturable component was sufficient to fit to DCF data giving similar values for K_m to those obtained by [1] (5.2±0.5μM and 5.3±1.5μM respectively), whilst V_{max} was approximately 3-fold higher (0.17±0.02nmol/min/10^6cells and 0.061±0.02nmol/min/10^6cells respectively). All the inhibition models investigated had K_i values similar to those published [1] (18.1±3.1μM) and similar Aikake Information Criteria values to each other, which gives a measure of the quality of each model. Model 2 and 3 had parameters more akin to that seen with DCF alone. In conclusion, this work demonstrates the successful application of mechanistic models to robustly estimate parameters for DCF kinetics and its mode of inhibition by gemfibrozil.

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Systems biology approaches to studying kinase signalling and the effects of drugs in the human platelet

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Platelets, small anucleate blood cells, play a vital protective function, aggregating to form a key component of blood clots that stop bleeding following vascular injury. In cardiovascular disease the same mechanism also triggers thrombosis which can lead to heart attacks and strokes. Whilst many of the molecules involved in the intracellular processes that underpin platelet activation and aggregation have been identified, their specific roles in determining the rate and extent of the exceptionally rapid platelet response have yet to be determined. Understanding these complex molecular mechanisms is important for development of more effective antithrombotic medicines. We present a systems biology approach where mathematical models that are direct representations of biological knowledge are compared to bespoke high density quantitative data. Our approach has enabled the generation of a mathematical model that describes the interplay between protein tyrosine kinases that underpin the role of platelets in hemostasis following activation by the collagen receptor GPVI. The model also incorporates experimental data using a bruton tyrosine kinase inhibitor, ibrutinib, that is known to cause bleeding in patients. Kinase inhibitors are increasingly utilized in cancer therapy and the model may help understand how kinases may be targeted without disrupting platelet function.

Discrete-Time L1 Adaptive Controller to Regulate In Vivo Protein Expressions

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The application of DNA nanotechnology to interface with cellular environment provides tremendous opportunities to expand the synthetic biological circuits. The current application of DNA nanotechnology spans smart therapeutics (Douglas et al, Science 2012), drug delivery (Perrault, Shih, ACS Nano 2014), imaging (Choi et al, ACS Nano 2014), and probes for cell biology (Shaw et al, Nat Methods 2014). The excellent programmability of nucleic-acid-based parts would enlarge the space of complex functionalities realized in synthetic biological circuits. Building on our earlier works on DNA strand displacement circuits to regulate DNA tweezers driven by transcriptional oscillators, we show how discrete-time L1 adaptive controller can be used to deliver drugs *in situ* in response to cellular condition. For this, we replace the model predictive controller used (Menolascina et al, PLOS CB 2014). Our controller automatically regulates the administration of inducer molecules to

the cells by comparing the actual protein expression level in the cell population with the desired expression level. We intend to use in the automated platform of (Menolascina et al, PLOS CB 2014) which is based on a microfluidic device, a time-lapse microscopy apparatus, and a set of motorized syringes, all controlled by a computer. They have tested the platform to force yeast cells to express a desired fixed, or time-varying, amount of a reporter protein over thousands of minutes. Here, the computer automatically switched the type of sugar administered to the cells, its concentration and its duration, according to the control algorithm. Our discrete-time L1 adaptive controller facilitates superior results on controlling expression of any protein, fused to a fluorescent reporter, provided that an external molecule known to (indirectly) affect its promoter activity is available.

Conceptually, our controller is also compatible to work with optogenetic systems that allow one to generate desired perturbations in the intracellular concentration of a specific protein in microbial cell culture. As light can be easily added and removed, this enables an easier dynamic control of protein concentration in culture than would be possible with long-lived chemical inducers. Implementation of this closed-loop control scheme is achieved by sampling individual cells from the culture apparatus, imaging and quantifying protein concentration, and adjusting the inducing light appropriately. The culturing apparatus can be operated as a chemostat, allowing one to precisely control microbial growth and providing cell material for downstream assays. Apart from the obvious applications in phenotype regulations, this method of specifically perturbing the concentration of a single protein and measuring the downstream signaling and transcriptional responses will allow experimentalists to make more informative perturbations to better elucidate the kinetics and architecture of biological networks for disease diagnosis and drug delivery.

Acknowledgment: Dr. Vishwesh Kulkarni is an Assistant Professor at the School of Engineering of the University of Warwick, and is affiliated with the Warwick Institute of Synthetic Biology, Warwick Medical Imaging Network, and Warwick Antimicrobial Resistance Centre. Fazul Islam is an MSc student at the School of Engineering, University of Warwick.

Establishing species-specific 3D liver microtissues for repeat-dose toxicology and advancing in vitro to in vivo translation through computational modelling

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Liver injury is a rising problem in the UK and in the US and over 50% of cases of acute liver failure (ALF) in the US are accounted for by adverse drug reactions (ADRs) (39% Paracetamol, APAP). APAP poisoning has become the most common cause of ALF in the UK and the USA.
Toxicity issues account for ~21% drug attrition during drug development, and liver toxicity is the most common reason for drug failure during the developmental process. Current 2D in-vitro model systems developed to assess ADRs and hepatotoxicity have a number of down falls and therefore, the emphasis on producing relevant and representative 3D models has therefore expanded in order to bridge the complexity gap between 2D systems and *in-vivo*.

3D cultures of hepatocytes *in-vitro* have recently emerged as a potentially better platform to recapitulate the *in-vivo* liver structure and to maintain long-term hepatic functions as compared with conventional 2D cultures. In the present study, we have used mathematical modelling to optimise a 3D hepatic spheroid system in terms of recapitulation of *in-vivo* liver oxygen gradients (13% - 4% O2) and we have used methods from porous media, multiphase modelling to predict compound penetration patterns within the spheroid as well as quantifying biomarker release for toxicity testing. The experimental model was constructed using the liquid-overlay technique in a multiwall format with freshly isolated primary rat hepatocytes. This method allows the formation of reproducible spheroids of varying cell seeding densities and provides a high-throughput platform for downstream investigation.

We demonstrate that these mathematically optimized primary rat spheroids display *in-vivo* characteristics including direct cell-cell contacts, bile canaliculi formation, cellular polarisation, 3D

cellular morphology and formation of secondary structures throughout the hepatosphere, suggesting an ideal platform for pharmacological and toxicological liver testing.

Estimation of Biliary Clearance of Drugs in Human Co-culture Hepatocytes using Mechanistic Simbiology Model

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The Hμrel *in vitro* cell culture system co-culturing human hepatocytes with non-parenchymal cells (eg stromal cells) in a controlled environment promotes bile canaliculi formation with polarised expression of transporter proteins. This system was evaluated to estimate kinetic rate constants for passive permeation, active uptake and biliary transport of model drugs (Pitavastatin, Rosuvastatin and Valsartan) by employing a holistic model constructed in Simbiology (V5, Mathworks, Inc) (Figure 1).

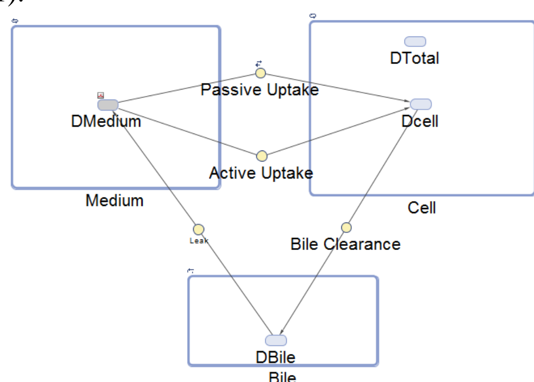

Figure 1. Simbiology Model for prediction of hepatic transport clearances

The concentration profiles in the different compartments were simultaneously fitted employing nonlinear regression using ODE15s (Stiff/NDF) to the individual values of total drug uptake into cells and in the bile compartment in presence and absence of transport and metabolic inhibitors . A covariate matrix was used to gauge the relationship between transport kinetic constants. Drug leakage from the bile compartment back into medium was modeled by constant 'K_{flux}' or variable 'K_{flux}' approaches. Table 1 summarises parameter estimates of transport kinetic constants for the three model drugs.

Parameter	**Pitavastatin**		**Rosuvastatin**		**Valsartan**	
	Constant K_{flux} Model	Variable K_{flux} Model	Constant K_{flux} Model	Variable K_{flux} Model	Constant K_{flux} Model	Variable K_{flux} Model
K_{mc} (min^{-1})	0.0063 (0.0027)	0.0063 (0.0027)	0.0012 (0.0006)	0.0012 (0.0006)	0.00027 (0.000025)	0.00027 (0.000025)
K_{cm} (min^{-1})	0.614 (0.227)	0.614 (0.227)	0.600 (0.207)	0.600 (0.207)	0.075 (0.045)	0.075 (0.045)
K_{mca} (min^{-1})	0.012 (0.012)	0.018 (0.006)	0.0011 (0.0002)	0.0049 (0.0003)	0.00059 (0.00005)	0.00075 (0.00008)
K_{cb} (min^{-1})	0.17 (0.22)	0.066 (0.076)	0.758 (0.044)	0.239 (0.0009)	0.042 (0.005)	0.056 (0.054)

Constant K_{flux}=0.044 min^{-1} (Pfeifer et al, 2015) Variable K_{flux}= 0.044 min^{-1} (0-5min), 3.5 min^{-1}(5-10min), 0.0046 min^{-1}(10-20min), 0.044 min^{-1} (20-25 min^{-1}) m=medium, c=cell, mca= medium-cell-active, cb=cell-bile n=2, Standard deviation in parenthesis.

The variable K_{flux} approach incorporates timed 'events' in Simbiology that reflect bile canaliculi contractions in vitro. It was concluded that employing the variable K_{flux} approach variability in estimates (K_{mca} and K_{cb}) was reduced by having better fit of observations.

Acknowledgement: This is a collaboration work funded by AstraZeneca (UK).