COPASI

2016 Conference on

Computational Modelling with COPASI

12-13 May, 2016
Manchester Institute of Biotechnology
Manchester, UK

PROGRAM
&
ABSTRACTS

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The University of Manchester
Manchester Institute of Biotechnology

BBSRC
bioscience for the future

10 YEARS 2006–2016
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Discovery through innovation

PROGRAM

Thursday, 12 May

9:15 AM	**Welcome**	Organizers		
9:30 AM	**Invited Talk 1**	Ines Heiland	*4*	chair: Stefan Hoops
10:15 AM	**Session 1**	Jens Hahn	*8*	chair: Norman Paton
10:35 AM		Sven Sahle	*9*	
10:50 AM		Vijayalakshmi Chelliah	*10*	
11:05 AM	Coffee Break	Atrium		
11:35 AM	**Session 2**	Adaoha Ihekwaba	*11*	Chair: Ettore Murabito
11:55 AM		Athraa Jani	*12*	
12:10 PM		Jennifer R. Chase	*13*	
12:25 PM	Lunch & posters	Atrium		
1:25 PM	**Invited Talk 2**	Carole Proctor	*5*	chair: Juergen Pahle
2:10 PM	**Session 3**	Mark McAuley	*14*	chair: Chris Redfern
2:30 PM		María Isabel González-Sánchez	*15*	
2:50 PM		Amy Morgan	*16*	
3:05 PM		Alvaro Martinez Guimera	*17*	
3:20 PM	Coffee Break	Atrium		
3:50 PM	**Session 4**	Emma Saavedra	*18*	chair: Douglas Kell
4:10 PM		Ina Pöhner	*19*	
4:25 PM		José Manuel Villalba	*21*	
4:40 PM		Philip Hall	*22*	
4:55 PM		Edelmira Valero	*23*	
5:10 PM	**Reception**	Atrium		

Friday, 13 May

9:15 AM	**Invited Talk 3**	Nicolas Le Novère	*6*	chair: Ursula Kummer
10:00 AM	**Session 5**	Megan Uttley	*24*	chair: Brian Klahn
10:20 AM		David Hodgson	*25*	
10:35 AM		Neil Mcdonald	*26*	
10:50 AM		Nadine Veith	*27*	
11:05 AM	Coffee Break	Atrium		
11:35 AM	**Session 6**	Ciaran Welsh	*28*	chair: Frank Bergmann
11:55 AM		Peyman Zarrineh	*29*	
12:10 PM		Chuan Fu Yap	*30*	
12:25 PM		Elaina Maldonado	*31*	
12:40 PM	**Closing**	Organizers		
12:50 PM	Pack lunch	Atrium		

Invited Talks

Tissue specific models of Trp- and NAD-metabolism — insights into metabolic crosstalk

Ines Heiland[1]

1 - The Arctic University of Norway, Tromsø, Norway

Keywords: metabolism, tryptophan, NAD-biosynthesis, metabolic control analysis, pipeline, python, script

We have in recent years built a comprehensive model of mammalian tryptophan metabolism and are in the process of doing the same for NAD-biosynthesis. Using these models we are able to simulated disease and tissues specific metabolic differences based on available expression data and previously measured kinetic parameters. To do this we have developed a COPASI-based pipeline that allows the calculation of metabolic changes for individual patients. Using metabolic control analysis we are furthermore able to predict potential intervention strategies and analyse tissue specific differences in the control of tryptophan metabolism. To perform these analysis we have developed a python library for the modification and analysis of models created with COPASI. Script based modifications of these models have also enabled us to analyse the metabolic crosstalk between different tissues, especially between brain and liver, two organs of specific interest with respect to tryptophan metabolism.

Computational modelling of musculoskeletal ageing

Carole Proctor[1]

1 - MRC/Arthritis Research UK Centre for Musculoskeletal Ageing (CIMA), Musculoskeletal Research Group, Institute of Cellular Medicine, Medical School, Newcastle University, UK

Keywords: ageing, musculoskeletal, osteoarthritis, bone homeostasis, cartilage, stochastic simulation, oxidised proteins

Age-related musculoskeletal diseases such as osteoarthritis, osteoporosis and sarcopenia are a major cause of morbidity in the elderly population. The MRC/Arthritis Research UK Centre for Integrated research into Musculoskeletal Ageing (CIMA) is a collaboration between the universities of Liverpool, Newcastle and Sheffield which was set up to develop an integrated approach to understand the mechanisms of ageing that affect musculoskeletal tissues. Since the molecular mechanisms involved are complex, we are using computational modelling to complement experimental work.

I will give an overview of the modelling work that is recently being carried out within CIMA and will then focus on three models, two which have been developed to investigate the mechanisms involved in osteoarthritis, and a third model of the molecular pathways involved in maintaining bone homeostasis. These models were constructed in a modular way using the Systems Biology Markup Language. Both deterministic and stochastic approaches were used and simulations and model analysis were carried out in COPASI.

The models were developed in collaboration with experimental scientists and a range of data were used to calibrate and validate the models. For example, histochemical and immunohistochemical data of cartilage showed many age-related changes including a decline in autophagy, an increase in apoptosis, and an increase in levels of oxidised proteins, matrix metalloproteinase-13 (MMP-13) level (an enzyme that is responsible for the pathological degradation of cartilage, a key feature of osteoarthritis). Since there was considerable cell-cell variability in the measured outcomes, we mainly used stochastic simulation to model age-related changes in cartilage. This model showed that the main source of this cellular heterogeneity is due to the stochastic nature of cellular damage, as most variability in the model output was seen in the levels of oxidized proteins. There was also considerable variability in the time at which damage starts to accrue, and this accounted for the observed wide variability in the activation of matrix degrading enzymes.

Calcium signalling

Nicolas le Novère[1]

1 - Babraham Institute, Cambridge, UK

Keywords: signalling, calcium, calmodulin, allostery, neuron, synaptic long-term potentiation, synaptic long-term depression

Common forms of short-term synaptic plasticities are associated with non-linear responses to calcium increases in the post-synaptic compartment. Calmodulin, one of the main calcium sensors in eukaryotic cells, is a small protein that carries four calcium binding sites with different affinities. Allostery and thermodynamic linkage explains many Calmodulin's properties, in particular the apparent increasing calcium affinity with fractional occupancy, the activity of non-saturated forms of calmodulin, and the increase in calcium affinity once calmodulin is bound to a target. Different conformations of calmodulin binds various targets with different affinities. These properties of calmodulin may suffice to explain the differential activation of calcineurin, leading to synaptic long-term depression, and calcium/calmodulin kinase II, leading to synaptic long-term potentiation. This also provides an explanation for the role of Neurogranin as a Calmodulin buffer, regulating availability of Calmodulin depending on calcium concentrations. Allosteric models can be embedded in models of biochemical pathways to study the kinetics of responses to calcium. Finally, such models can be integrated with electrophysiological models at the level of the entire neuron.

Contributed Talks

From pieces to the whole — Concepts for modular modelling of yeast

Jens Hahn[1], Stefan Forgo[1], Jorin Diemer[1], Katja Tummler[1], Tom Altenburg[1], Stephan O. Adler[1], Judith Wodke[1], Max Schelker[1], Thomas W. Spiesser[1], Ulrike Münzner[1], Friedemann Uschner[1], Sebastian Thieme[1], Ana Bulović[1], Paula Martinell[1], Marcus Krantz[1], Martin Seeger[1], Max Flöttmann[1], Jannis Uhlendorf[1], Edda Klipp[1]

1 - Humboldt-Universität zu Berlin,Germany

Keywords: Saccharomyces cerevisiae, Modelling, ODE

A large number of detailed high quality models of specific cellular processes have over the last years proven to be valuable for the understanding of biological functionality. However, they disregard interactions with other intracellular processes and the embedding in higher order processes, such as cell cycle, volume variation, and environmental conditions. The integration of those functionalities of different levels of cell granularity and time-scales is itself technically challenging.

We present a first approach to solve these issues by combining a model of yeast cell cycle and gene regulation in the context of a changing cell size and minimal metabolism. The cell cycle model includes the key cyclins and their kinases, whose expression is controlled by the gene expression model. These models are embedded in a biophysical description of the cell physiology and coupled to a coarse-grained carbon metabolism to include sensitivity to nutrient availability.

To combine and simulate the different modules, we developed a software environment that specifically tackles the challenges of integrating biological models with conceptually different mathematical formulations, time-scales, and resolutions. The framework is written in Python and provides several numerical solvers, SBML support and a COPASI interface.

Our approach focuses on the interfaces between biological processes represented in different modules. The modules themselves can be modified independently and easily exchanged within the framework. We provide a comprehensive and reproducible method to define and characterise interfaces between different modules and use these interfaces to simulate models together, even if they rely on different modelling approaches.

A new efficient approach to fit stochastic models on the basis of high-throughput experimental data using a model of IRF7 gene expression as case study

Luis Aguilera[1], Christoph Zimmer[1], Sven Sahle[1], Ursula Kummer[1]

1 - Heidelberg University, Germany

Keywords: stochastic models, parameter estimation, IRF7 gene expression

Mathematical models are used to gain an integrative understanding of biochemical processes and networks. Commonly the models are based on deterministic ordinary differential equations. When molecular counts are low, stochastic formalisms like Monte Carlo simulations are more appropriate and well established. However, compared to the wealth of computational methods used to fit and analyze deterministic models, there is only little available to quantify the exactness of the fit of stochastic models compared to experimental data or to analyze different aspects of the modeling results. Here, we developed a method to fit stochastic simulations to experimental high-throughput data, meaning data that exhibits distributions. The method uses a comparison of the probability density functions that are computed based on Monte Carlo simulations and the experimental data. Multiple parameter values are iteratively evaluated using optimization routines. The method improves its performance by selecting parameters values after comparing the similitude between the deterministic stability of the system and the modes in the experimental data distribution. The programming strategy involved the binding of COPASI with Matlab to generate a fully automated source code. As a case study we fitted a model of the IRF7 gene expression circuit to time-course experimental data obtained by flow cytometry. IRF7 shows bimodal dynamics upon IFN stimulation. This dynamics occurs due to the switching between active and basal states of the IRF7 promoter. However, the exact molecular mechanisms responsible for the switching of the IRF7 promoter state are not fully understood. Our results allow us to conclude that the activation of the IRF7 promoter by the combination of IRF7 and ISGF3 is sufficient to explain the observed bimodal dynamics.

BioModels: a public model sharing resource for computational models of biological/biomedical systems

Vijayalakshmi Chelliah[1], Nick Juty[1], Mihai Glont[1], Nicolas Le Novère[2], Henning Hermjakob[1]

1 - EMBL - European Bioinformatics Institute, UK; 2 - Babraham Institute, UK

Keywords: BioModels, mathematical modelling, resource, database, mechanistic models, biological process, COPASI

Mechanistic models are becoming one of the cornerstones of the computational life sciences. Coupled with high-throughput 'omics' and network analysis, hypothesis design and the use of predictive models is becoming standard practice to understand the mechanisms underlying complex biological systems.

BioModels [1, 2] is an invaluable core resource for the systems biology/pharmacology community. It stores a vast collection of the literature-based mechanistic models in standard formats, many of which are physiologically and pharmaceutically relevant, and describe a wide range of biological processes at different biological scales. These models can also serve as a comprehensive body of knowledge on existing processes or be used as building blocks, facilitating the repurposing of a model through further development, refinement or merging.

BioModels provides two sets of models: (i) models described in scientific literature, and (ii) models generated from pathway resources (path2models [3]). The components, structure and behaviour of a large proportion of these models are manually verified ensuring correspondence to the original reference publication. This verification process is conducted using a tool other than that used in the original publication, thus precluding tool specific errors or hidden dependencies; the simulation results of more than 60% of the curated models have been verified using COPASI.

The model elements are cross-referenced (annotated) to records from external database resources to precisely relate them to the corresponding biological processes or physical entities they represent. Over 40 external database resources and ontologies are used to cross-reference the model components. This facilitates efficient search and retrieval of the models from the database, and also helps in model comparison, merging and expansion with novel information.

BioModels is accessible through a web interface and programmatically through web services. Hosted models are freely available for use, modification and redistribution to all users under the terms of Creative Commons CC0.

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3. Büchel et al. (2013). Path2Models: Large-scale generation of computational models from biochemical pathway maps.BMC Systems Biology. 7:116.

Computational modelling and analysis of the molecular network regulating sporulation initiation in *Bacillus subtilis*

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1 - Institute of Food Research, UK; 2 - Los Andes University, Colombia

Keywords: Systems biology, Computational modelling, Sensitivity analysis, Signal transduction, Sporulation, *Bacillus subtilis*

BACKGROUND: Bacterial spores are important contaminants in food, and the spore forming bacteria are often implicated in food safety and food quality considerations. Spore formation is a complex developmental process involving the expression of more than 500 genes over the course of 6 to 8 hrs. The process culminates in the formation of resting cells capable of resisting environmental extremes and remaining dormant for long periods of time, germinating when conditions promote further vegetative growth. Experimental observations of sporulation and germination are problematic and time consuming so that reliable models are an invaluable asset in terms of prediction and risk assessment. In this report we develop a model which assists in the interpretation of sporulation dynamics.

RESULTS: This paper defines and analyses a mathematical model for the network regulating *Bacillus subtilis*sporulation initiation, from sensing of sporulation signals down to the activation of the early genes under control of the master regulator Spo0A. Our model summarises and extends other published modelling studies, by allowing the user to execute sporulation initiation in a scenario where Isopropyl β-D-1-thiogalactopyranoside (IPTG) is used as an artificial sporulation initiator as well as in modelling the induction of sporulation in wild-type cells. The analysis of the model results and the comparison with experimental data indicate that the model is good at predicting inducible responses to sporulation signals. However, the model is unable to reproduce experimentally observed accumulation of phosphorelay sporulation proteins in wild type *B. subtilis*. This model also highlights that the phosphorelay sub-component, which relays the signals detected by the sensor kinases to the master regulator Spo0A, is crucial in determining the response dynamics of the system.

CONCLUSION: We show that there is a complex connectivity between the phosphorelay features and the master regulatory Spo0A. Additional we discovered that the experimentally observed regulation of the phosphotransferase Spo0B for wild-type B. subtilis may be playing an important role in the network which suggests that modelling of sporulation initiation may require additional experimental support.

Estimate the Number of Nodes in a Nanonetwork

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1 - University of Liverpool, UK

Keywords: Nanonetworks, Quorum Sensing, molecular communication, diffusion, synchronization

Nanotechnology provides promising solutions to problems in different disciplines. A nano-machine is the basic functional unit of any nano-system. When we have more than one nano-machine, the interaction between these machines would form a nanonetwork.

We consider a model of a nanonetwork consisting of N nano-machines, which are located in a space, and communicating between each other via diffusion. The goal of the model is to estimate N by certain devices in the nanonetwork, which can be done by adopting the mechanism of quorum sensing. Quorum sensing is a biological process that enables the synchronization of a population of bacteria. In order to synchronize with the group, each bacterium releases a particular type of molecules at a constant rate. The concentration of that type of molecules in the environment increases proportionally with the bacterial population. By this way, bacteria are able to sense their population density by detecting the level of that certain type of molecules. Thus, we are inspired by this biological mechanism to obtain the main objective of this model.

Modeling of uterine glycogen metabolism of the mink

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1 - Northwest Nazarene University, USA

Keywords: glycogen, metabolic model, COPASI, hormonal regulation

In addition to the well-known liver and muscle, the mammalian uterus also stores glycogen. The apparent role of glycogen in this tissue is to serve as a supply of glucose for the endometrium, where the sugar is an energy source for embryos before the development of the placenta. In animals with obligate embryonic diapause, the pre-implantation time can be quite extended, (e.g., up to 60 days post coitus in the mink, *Neovison vison*). In the uterus glycogen metabolism under regulation by ovarian cycle hormones (estrogen and progesterone derivatives) in addition to insulin, all with very long cycle times for the hormones and glycogen levels. Very little of this regulation and metabolism is understood for the uterus. We have developed a small metabolic and regulatory model of glycogen metabolism for uterine tissue in COPASI 4.16 (Build 104), with 30 species and 10 elementary modes. The Parameter Estimation function of COPASI was used to fit glycogen synthesis and hormone interaction data for mink and cultured mink uterine cells. Glycogen phosphorylase levels were modeled to vary in response to progesterone, thus increasing glycogen catabolism during diapause and pregnancy relative to estrus. This model will guide future *in vitro* studies to understand the regulation of uterine glycogen metabolism, as well as *in silico* efforts to understand the bases of some human infertility due to poor embryonic implantation.

In silico simulation of chronic oxidative stress interference with redox signalling

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1 - University of Chester, UK; 2 - Liverpool Hope University, UK

Keywords: Folate Cycle, Ageing, DNA Methylation, Microbial Computational Model

Dietary folates (Vitamin B9) have a key role to play in health as deficiencies in the intake of these B vitamins have been implicated in a wide variety of clinical conditions. Folates function as single carbon donors in the synthesis of methionine and nucleotides. Moreover, folates have a vital role to play in the epigenetics of mammalian cells by supplying methyl groups for DNA methylation [1]. In mammalian cells folate metabolism is characterised by the complex biochemical reactions of the folate cycle. Microbial folate metabolism differs from its mammalian counterpart, as microbes have retained a biosynthetic pathway within the intracellular folate cycle. This pathway acts like a switch when environmental folate levels are low. To deal with the inherent complexity associated with both biochemical systems we are using computational modelling [2]. Our microbial kinetic model is the first of this system. We have used the model to help identify potential novel antifolates. Moreover, our COPASI formulated model supports the hypothesis that a folinic acid biosynthesis loop acts as a folate-mediated regulatory circuit in cell growth [3]. Our mammalian computational model of folate metabolism integrates with the biochemical reactions which underpin the DNA methylation cycle. Our biological rationale for doing this is unpinned by the knowledge that perturbations to the folate cycle are strongly coupled with age associated aberrant DNA methylation [4]. This suggests a deeper mechanistic understanding of the interaction between the folate cycle and DNA methylation could be pivotal in improving our understanding of ageing.

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2. Mooney, K.M., A.E. Morgan, and M.T. Mc Auley, Aging and computational systems biology. Wiley Interdisciplinary Reviews: Systems Biology and Medicine, 2016. 8(2): p. 123-139.
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Catalase-like activity of hemoproteins involved on oxidative stress: Kinetic study and computer simulation

María Isabel Gonzalez-Sanchez[1], José Manuel Villalba[1], Hermenegilda Macia[1], Ramón Varón[1], Edelmira Valero[1]

1 - University of Castilla-La Mancha, Spain

Keywords: Hydrogen peroxide, Hemoproteins, Oxidative stress, COPASI, simulation

Catalase-like activity of hemoproteins has a great physiological importance because it contributes to eliminate the excess of hydrogen peroxide, an oxygen reactive species whose high concentration in biological organisms can be very dangerous. Hydrogen peroxide triggers redox cycles with these proteins, leading to protein inactivation and oxygen evolution. In the present work, the catalase-like oxygen production by a hemoprotein in the presence of hydrogen peroxide was kinetically characterized with a Clark-type electrode. By considering all the experimental data obtained, a possible mechanism was proposed, including: (a) competition between the one-electron and the two-electron reductions of the oxoferryl free radical species of the hemoprotein, giving rise to the ferryl state and the met state of the protein, respectively; (b) competition between the superoxide-dependent inactivation of the protein and its reduction back to the met state. Computer simulations of the model were performed by numerically integrating the differential equations set describing the mechanism using COPASI 4.7 software, which was seen to yield predictions of the kinetic parameters variation consistently with the kinetic behavior experimentally observed. The apparent inactivation constants and partition ratio calculated were in agreement with the theoretical results obtained by computer simulation. We suggest that the catalase-like activity of hemoproteins must predominantly be a biocatalytic reaction that protects the protein against hydrogen peroxide induced suicide inactivation. Among the enzymes and proteins that could follow this mechanism are peroxidases, catalases and cytochrome c, which are very important in the defense of the biological organisms against the oxidative stress.

Computationally modelling the dynamics of cholesterol metabolism and ageing

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1 - University of Chester, UK; 2 - Edge Hill University, UK

Keywords: Cholesterol, Ageing, Cardiovascular Disease

Cardiovascular disease (CVD) accounted for 27% of all deaths in the United Kingdom in 2014, and was responsible for 1.7 million hospital admissions in 2013/14. This condition becomes increasingly prevalent with age, affecting 34.1 and 29.8% of males and females over 75 years of age respectively in 2011 (1). The dysregulation of cholesterol metabolism with age, often observed as a rise in low density lipoprotein cholesterol (LDL-C), and decline in high density lipoprotein cholesterol (HDL-C), has been associated with the pathogenesis of CVD (2). To compound this problem it is estimated that by 2050, 22% of the world's population will be over 60 years of age, while resistance to pre-existing cholesterol regulating drugs such as statins has also been observed (3). Therefore, it is apparent that research into additional therapies for CVD prevention is a growing necessity. However it is imperative to recognise that this complex system cannot be studied using a reductionist approach, rather its biological uniqueness necessitates a more integrated methodology. The systems biology paradigm provides a more holistic framework for conducting investigations of this nature (4). Therefore, we have adopted this approach, and used COPASI to investigate the dysregulation of whole-body cholesterol metabolism with age. This kinetic model builds on our previous work in this area by including a more mechanistic representation of cholesterol absorption and biosynthesis (5). Using our model we were able to investigate the impact of intrinsic aging on cholesterol metabolism and were able to determine how dietary perturbations affect LDL-C and HDL-C levels (6). In the future it is hoped that the findings from our approach will inform novel nutrient and pharmacological based interventions which may help prevent CVD.

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In silico simulation of chronic oxidative stress interference with redox signalling

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1 - Newcastle University, UK

Keywords: systems modelling, Nrf2 signalling, oxidative stress, skeletal muscle

Redox signalling underlies a number of beneficial responses triggered by skeletal muscle during a period of exercise. Aged skeletal muscle is associated with a state of damage and oxidative stress where the protective responses triggered by an exercise stimulus are blunted. However, it is mechanistically unclear if oxidative stress is the direct causative agent of the impaired redox signalling. The elucidation of the nature of this signalling dysfunctionality in skeletal muscle is a step towards improving the efficacy of exercise as a lifestyle intervention for the elderly.

Numerous ordinary differential equation (ODE) -based models have been constructed in an effort to understand the behaviour of redox signalling networks. The ability of these models to reproduce experimental data in a variety of biological settings argues for the employment of such methods to integrate experimental data into a coherent in silico physicochemical framework that aids the interpretation of such data and the generation of new hypotheses.

In this work we employ COPASI to create a simple kinetic model of Nrf2 signalling, which we calibrate with experimental data derived from C2C12 myotubes. We further explore *in silico* potential mechanisms of oxidative stress interference with signalling within the network.

Kinetic modeling of the antioxidant metabolism in *Trypanosoma cruzi*: In search of therapeutic targets

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1 - Instituto Nacional de Cardiologia, Mexico

Keywords: antioxidant metabolism, glutathione, trypanothione, parasite, drug target, Trypanosoma

BACKGROUND: *Trypanosoma cruzi* is the protist parasite that causes human American trypanosomiasis, a disease endemic of Latin American countries. In trypanosomatids, the trypanothione, a conjugate of two glutathione and one polyamine moieties, is the main antioxidant metabolite; hence the trypanothione-based antioxidant enzymatic machinery replaces the function of the glutathione system in mammals. The aim of this work is to identify the enzymes that mainly control the trypanothione metabolism in *Trypanosoma cruzi* by applying the quantitative analyses of kinetic modeling and the fundamentals of Metabolic Control Analysis.

METHODS: Kinetic models of the trypanothione-synthesis and trypanothione-dependent peroxide detoxification pathways were constructed using the GEPASI/COPASI platform using (i) the kinetic parameters of the recombinant pathway enzymes determined under near-physiological conditions, and (ii) the enzyme activities in the cells. Model validation was established by its ability to simulate the metabolite concentrations and fluxes of the in vivo pathways. The in silico predictions regarding the pathway flux-control distribution were evaluated in the parasites in supplementation experiments with thiol-molecules and polyamines.

RESULTS: The models could robustly simulate the fluxes and metabolite concentrations found in the parasites. The models indicated that gamma-glutamylcysteine synthase > trypanothione synthase >>> polyamine supply were the main controlling steps of trypanothione synthesis. Supplementation of parasites with cysteine and GSH, but not with spermidine or putrescine, increased the trypanothione pool which was in agreement with the flux-control distribution obtained by modeling. In the trypanothione-peroxide detoxification system, tryparedoxin was the main controlling enzyme, which is in agreement with pathway reconstitution data that indicated the tryparedoxin/tryparedoxin-peroxidase redox pair fully controlled the pathway flux, with negligible control exerted by trypanothione reductase.

CONCLUSIONS: The most controlling steps of the trypanothione metabolism in *T. cruzi* were identified. The results indicated that inhibition of either gamma-glutamycysteine synthetase, trypanothione synthetase or tryparedoxin will have much stronger adverse effects on the parasite antioxidant defense than inhibition of low-controlling enzymes such as trypanothione reductase, a popular protein for drug-target studies.

Insight for anti-parasitic drug design — From comparative pocketome analysis to computational modeling of a parasitic folate & biopterin pathway

Ina Pöhner[1], Joanna Panecka[1], Francesca Spyrakis[2], Talia Zeppelin[1], Maria Paola Costi[2], Rebecca C. Wade[1,3]

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Keywords: Neglected tropical diseases, Folate pathway, dihydrofolate reductase, DHFR, pteridine reductase 1, PTR1, pocket analysis, pathway modeling

Trypanosomatid parasites are the etiological agents of several neglected tropical diseases (NTD) including sleeping sickness, Chagas' disease and leishmaniasis which collectively affect nearly 10 million people worldwide. The few available therapeutics are characterized by toxicity, poor efficacy and emerging parasite resistance, implying a need for new, safe and effective drugs. Drug discovery for NTD often involves target-based approaches, for example focusing on the folate pathway as an established target pathway in the treatment of bacterial infections and some parasitic diseases, such as malaria. Validated key targets include dihydrofolate reductase (DHFR), which is the target of classical antifolate drugs like methotrexate (MTX). MTX is however ineffective against Trypanosomatids due to pteridine reductase 1 (PTR1), an enzyme mostly responsible for the salvage of pterins. PTR1, which has in part overlapping activity with DHFR, can provide a metabolic bypass supplying reduced folates necessary for parasite survival in the case of DHFR inhibition.

Consequently, PTR1, which is unique to the parasite, is considered a promising target for the development of improved therapies. However, many PTR1 inhibitors alone seem incapable of parasite growth inhibition and drug design efforts focus on the development of dual inhibitors targeting both PTR1 and DHFR as well as combination therapies with MTX, which was shown to improve the efficacy and the potency of PTR1 inhibitors.

Aiming at the identification of binding site features common to PTR1 and DHFR to be utilized in the design of dual inhibitors, we carried out an extensive comparison of the structural and physico-chemical properties of folate pathway enzyme binding pockets. We found a surprisingly low similarity of the molecular binding features of PTR1 and DHFR, despite their overlapping substrate pool. DHFR binding pockets were clearly more hydrophobic than PTR1 pockets. As a consequence, the parasitic DHFR appears more druggable than the key target PTR1, but its closely related human homolog complicates efforts to achieve dual inhibition. Our analysis pinpoints both potential sites for optimization towards the parasitic targets and differences to allow for off-target selectivity.

Beyond the well-studied examples of PTR1 and DHFR, the parasites utilize a complex network involving many additional overlapping enzyme functionalities. Therefore, we expanded our initial

studies focused on the experimentally best characterized proteins to involve the full pocketome of the Leishmania major folate/biopterin pathway. Mathematical modeling of the parasitic folate and biopterin metabolism will supplement the structure-based studies by simplifying the identification of potential additional promising points of attack in the network, allowing the estimation of minimum levels of enzyme inhibition, and adding to our understanding of target-crosstalk and the effects observed in combination therapy by PTR1 and known DHFR inhibitors.

Computer-aided simulation of the enzymatic synthesis of *o*-diphenolic compounds using tyrosinase

José Manuel Villalba[1], María Isabel Gonzalez-Sanchez[1], Mª Emilia Cambronero[1], Edelmira Valero[1]

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Keywords: COPASI, tyrosinase, *o*-diphenolic compounds, simulated progress curves, nonlinear set of differential equations

Catechol derivatives are present in numerous natural products and synthetic compounds used in various sectors of the chemical industry such as food, cosmetic, pharmaceutical and polymer industries. The antioxidant activity usually conferred to these compounds is the property on which their application is often based. Although several chemical methods are available to chemists to produce catechols, the enzymatic ortho-hydroxylation using tyrosinase in the presence of an excess of a reducing agent, such as ascorbic acid or NADH, and molecular oxygen constitutes one of the most practical approaches as long as the ortho-selectivity of the process can be controlled, in a nonpolluting way. Examples of *o*-diphenolic compounds that can be thus synthesized include oleuropein, hydroxytyrosol, 3,4-dihydroxyphenylacetic acid, L-DOPA and 3'-hydroxyacetaminophen. In the present communication, a mathematical model of the system is proposed, based on the internal catalytic action mechanism of tyrosinase. Simulated progress curves of the biocatalytic reaction were obtained by numerical solution of the nonlinear set of differential equations (9 eqs) corresponding to the model proposed by using COPASI. This model is valid for simulating the kinetic behavior of the tyrosinase-mediated synthesis of any o-diphenol from its corresponding monophenol in the presence of a reducing agent, the only modification necessary being the numerical values of the rate constants. Therefore, the present model can be helpful for implementation of the biocatalytic process for industrial purposes, permitting the process to be controlled.

A systems modelling study of the integrated stress/damage response and nutrient signalling network

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Keywords: Systems Biology, Ageing, Disposable Soma, Nutrient Allocation, ROS, IGF Signalling, AKT, FOXO3a, mTOR

The disposable soma theory predicts that levels of maintenance and repair are influenced by resource availability. The interplay between the FOXO-Sestrin-p53 signalling network regulating stress and damage response and the IRS1-Akt-mTOR-AMPK nutrient sensing network monitoring resource availability offer an opportunity to further our understanding of how this influence is achieved. These two signalling systems are deeply embedded with complex interactions (such as AKT and MDM2, pTEN and PIP3 and FOXO with AMPK and TSCs) involving multiple feedbacks which govern cellular outcome such as growth arrest, apoptosis, senescence or proliferation. This project proposes to build on and extend our current experimental and computational work to develop a well parameterised dynamic computational model of this integrated network.

FOXO is a well characterised protein which affects cellular growth, division and apoptosis in response to DNA damage signals. It achieves this due to its activity as a transcription factor for many genes including those for AMPK, Rictor, Sestrins and many others within the nutrient sensing network. FOXO is however regulated by the Insulin signalling pathway via AKT phosphorylation. AMPK activates TSC complex to inhibit mTOR complex 1 in response to energy stress and inhibits growth whilst encouraging autophagy. It is also directly and indirectly regulated by p53 and other damage response elements. It is these elements amongst others that are important for the investigation of this interplay between nutrients and stress. However there is division about how the nutrient signalling pathways, such as Insulin like pathway, are affected by chronically stressful conditions, such as a high redox environment. Some suggest that, over an extended period, ROS actually works to maintain transcription (contrary to established view) in order to maintain the levels of repair proteins and allow for a high protein turnover to remove damaged components. The application of an integrative systems biology approach is ideally suited to disentangling the complexity of the interaction and we will use this to address divergent views as outlined above.

Using the tools developed in our laboratories, we generated the data and models required which allow us to test predictions on cell fate in both stress/damage response and nutrient sensing networks. These developments were then combined to examine differences in physiological state in response to combinatorial effects of altered nutrient levels, environmental stress and perturbed intracellular processes.

Plant metabolic modeling: the ascorbate-glutathione cycle in chloroplasts under light/dark conditions

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Keywords: Light/dark cycles, Ascorbate-glutathione cycle, Computer simulation, Oxidative stress, Reactive oxygen species, Chloroplast

Light/dark cycles are probably the most important environmental signals that regulate plant development. Light is essential for photosynthesis, but an excess, in combination with the unavoidable presence of atmospheric oxygen inside the chloroplast, leads to excessive reactive oxygen species production. Among the defense mechanisms that activate plants to cope with environmental stress situations, it is worth noting the ascorbate-glutathione cycle, a complex metabolic pathway in which a variety of photochemical, chemical and enzymatic steps are involved. We herein studied the dynamic behavior of this pathway under light/dark conditions and for several consecutive days. For this purpose, a mathematical model was developed with a variable electron source whose flux is directly proportional to the intensity of solar irradiance during the photoperiod, and which is continuously turned off at night and on again the next day. The model is defined by a nonlinear system of ordinary differential equations with an on/off time-dependent input, including a parameter to simulate the fact that the photoperiod length is not constant throughout the year, and which takes into account the particular experimental kinetics of each enzyme involved in the pathway. Unlike previous models, which have only provided steady-state solutions, the present model is able to simulate diurnal fluctuations in the metabolite concentrations, fluxes and enzymatic rates involved in the network. Numerical integration was performed with the help of the COPASI 4.7 software (Build 34) using a deterministic algorithm (LSODA) that is able to deal with stiff ODEs. The resulting ODE model (15 days) consists in 13 species and 67 global quantities (kinetic parameters, enzymatic rates and fluxes). The obtained results are broadly consistent with experimental observations and highlight the key role played by ascorbate recycling for plants to adapt to their surrounding environment. This approach provides a new strategy to in vivo studies to analyze plant defense mechanisms against oxidative stress induced by external changes, which can also be extrapolated to other complex metabolic pathways to constitute a useful tool to the scientific community in general.

Modelling the network of bioactive lipid mediators in human skin cells

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Keywords: Skin, Eicosanoids, Inflammation, Computational Modelling, Medical Systems Biology

Inflammatory responses in the skin play a major role in a number of pathophysiological conditions such as sunburn, dermatitis and psoriasis. A major contributor to the regulation of inflammation is a class of potent bioactive lipid mediators, known as the eicosanoids. Eicosanoids are derivatives of the polyunsaturated fatty acid arachidonic acid. The family of eicosanoids includes more than 100 bioactive lipid species, including prostaglandins, leukotrienes, thromboxanes and prostacyclin. These mediators are known to be involved in various stages of the inflammatory response, and their biochemistry has been targeted for the development of therapeutics, including non-steroidal anti-inflammatory drugs (NSAIDs). Here, we introduce a mechanistic mathematical model of the network of cutaneous eicosanoids that includes multiple substrates and enzyme isoforms. This kinetic model of lipid mediator dynamics will be analysed using an efficient Monte Carlo ensemble modelling method to explore the landscape of potential model behaviours. The results of the study will allow for more targeted experiments to be designed, will permit a detailed mapping of the lipid networks contributing to skin inflammation, and ultimately will support the design of new interventional strategies to combat skin disease.

Investigating the changes in TGF-β signalling with age using a systems biology approach

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Keywords: TGF-β, Osteoarthritis, systems biology, inflammation

Osteoarthritis (OA) is a degenerative condition that is caused by the dysregulation of many molecular signalling pathways. TGF-β is a cytokine with pluripotent effects on OA development, initially protecting against inflammation driven damage, whilst promoting the restoration of cartilage. However, as we age TGF-β promotes induction of catabolic enzymes causing excessive damage to cartilage. A change in the ratio of two TGF-β type one receptors has been shown, the shift correlating with the spontaneous development of OA in mice models. Dr Carole Proctor created a model in copasi to show the stochastic changes in TGF-β signalling during ageing. We aim to expand this model incorporating how these changes affect the cells response to an inflammatory stimulus. Using a chondrocyte specific cell line (SW1353) in combination with this modelling based approach we aim to identify the key changes in the pathway that turn TGF-β from a protective to destructive cytokine.

Using Copasi to model the effect of protein stoichiometry on the unfolded protein response in cancer cells

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Keywords: Unfolded protein response, ER stress, Cancer, Dynamic modelling

Increase in cellular stress levels leads to an increase in the amount of unfolded proteins (UFP) within a cell and initiates a protective mechanism, the unfolded protein response (UPR). The UPR consists of three distinct networks that control the level of UFP within the cell by regulating pro-autophagic and, under irresolvable stress, pro-apoptotic mechanisms. The UPR is dependent upon interactions between GRP78 and the three activators, IRE1, PERK and ATF6; perturbations in these interactions are implicated in various diseases including cancer. Work carried out in Melanoma, Glioblastoma and Neuroblastoma cell lines revealed that the stoichiometry of the three activators varies between cell types and has a significant impact upon the sensitivities of different cell lines to the proteasome inhibitor Bortezomib. We have used Copasi to develop a dynamic model of the UPR, focusing on the interactions between the three activating proteins and their inhibitor GRP78. Employing experimental data for the downstream effectors of each of the three activators, parameter estimation was carried out for three different cell types and six different cell lines. The model predicted the outputs for the downstream effectors of each branch of the UPR in both of the melanoma and glioblastoma cell lines as well as one of the neuroblastoma cell lines. The model was validated using GRP78 overexpression data. Initial concentrations for IRE1, ATF6, PERK and GRP78 were adjusted to reflect experimental data for cells over-expressing GRP78. After four hours of simulation, the model output replicated the experimental data at the relevant time points. This model will facilitate a better understanding of the UPR in cancer and reveal novel targets for biomarker discovery and drug development.

Combining dynamic modelling and quantitative proteomics

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Keywords: ODE-based dynamic modelling, parameter estimation, quantitative proteomics, lactic acid bacteria, primary energy metabolism

The lactic acid bacterium, Enterococcus faecalis, relies almost exclusively on glycolysis and fermentation for energy production. This suggests that targeting its primary energy metabolism may lead to perturbation of cell growth and proliferation. In this study, we investigated the primary energy metabolism of this opportunistic human pathogen. First, we constructed an ODE-based dynamic model to describe the glucose uptake, glycolysis, fermentation and export of fermentation "end-"products using COPASI. As kinetic parameters are only sparsely available for this organism, we derived unknown kinetic constants by fitting the model to experimentally determined metabolite concentrations using parameter estimation. The metabolite data comprised selected extra- and intracellular metabolites measured in a time-course experiment after a glucose pulse.

To better understand the primary energy metabolism of Enterococcus faecalis, we combined dynamic modelling with quantitative proteomics. We used a mass spectrometric technique, PCT-SWATH, to measure proteomic changes during the glucose pulse. Using spiked-in synthetic peptides of known concentrations, we quantified selected "key-"enzymes of the primary energy metabolism in absolute concentrations during the time-course experiment and observed intriguingly high enzyme concentrations. In order to integrate the dynamic changes of enzyme abundances, we further constructed a small model describing phenomenologically the gene expression and protein biosynthesis of the quantified "key-"enzymes. The model parameters were derived by parameter estimation while fitting the model to dynamic changes of the measured enzyme abundances.

Finally, we combined the two models. First analyses of the extended model suggests that processes that are directly involved in glucose uptake are most sensitive.

Pydentify: A python module for performing identifiability analysis using COPASI

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Keywords: Parameter Estimation, Identifiability Analysis, Profile Likelihood, Python Module

Parameter estimation is a difficult problem in systems modelling owing to high dimensionality and largely unknown topology of the systems being modelled. Identifiability analysis is a necessary part of parameter estimation to assess whether parameters can uniquely be determined by the defined optimization problem. It is not however always simple and straight forward to perform an identifiability analysis. The current work extends the work of Schaber (Biosystems 2012), which provides a method to calculate the profile likelihood method of identifiability analysis using the popular GUI based simulation engine, COPASI. The current work provides a tool that automates this procedure. Moreover the current work extends this method to facilitate the calculation of multiple profile likelihoods from an arbitrary number of parameter sets, indexed by rank of best fit. Lastly, facilities are provided to plot the results of the profile likelihood calculations and to automate the calculation of the associated likelihood ratio based confidence intervals. The use of the software is demonstrated using published models. Overall this work provides a tool to facilitate the estimation of parameters in systems biology using COPASI.

Managing Systems Biology Models Throughout their Lifecycle

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Keywords: Model Provenance, Model Versioning, Undo framework, COPASI

Modelling and simulation is an approach at the heart of systems biology research, which integrates theory, modelling, and experiments to provide a system-level understanding of biological processes. Although large numbers of models are becoming available in public repositories like the BioModels database or the Physiome model repository, there is almost no information available about the development process of those models.

We are implementing a comprehensive set of features in COPASI to allow tracking and management of the model development process. These features will result in automatic capture of the model development history and allow the user to keep snapshots of arbitrary versions along that process. An "undo" framework has been implemented to capture within-session edits, allowing users to undo and redo their actions on model entities. Similarly, a "provenance" framework has been implemented to keep track of all inter-session changes to the model. The provenance is serialized using the W3C standard PROV-XML. A provenance log browsing interface allows COPASI users to inspect the entire history of the development of a model. A "versioning" framework has also been developed to allow users to create and store versions of the model. These versions are included as part of the model and can be restored or deleted at any stage of development process. We are making use of the COMBINE Archive format to store the main model, all its user-defined versions, and the provenance log.

We have implemented the proposed model management facilities in C++. These have been integrated into COPASI. COPASI is freely available for download at http://copasi.org.

Towards automated construction of kinetic metabolic models with GRaPe.

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Keywords: Metabolic Modelling, Systems Biology, Parameter Estimation, Kinetic Model, Dynamic Model

The construction of kinetic models of metabolic pathways has always been hindered by the limited availability of kinetic parameters, in addition to incomplete knowledge on the reaction mechanisms. Strategies have been developed to allow the generation of kinetic models with limited information. Despite this, not many large-scale dynamic and integrative models have been generated. The aim of this research is to streamline the process of generating large-scale metabolic models, while using metabolomic, proteomic and gene expression data to inform parameter values. The use of aforementioned integrative data in model construction would greatly enhance the parameter estimation process, reducing redundancy in parameters and thereby increasing the model's predictive capability.

Previously, the GRaPe tool was developed in order to streamline the construction of metabolic models through automated generation of kinetic equations. However a number of limitations affected the performance of the tool, which are now being addressed in this project. First, convenience kinetics has been introduced to replace the previously used reversible Michaelis-Menten rate equations. Convenience kinetics requires fewer parameters, which reduces the burden on parameter estimation for the models. Additionally, it allows for inclusion of modifiers such as activators into model building. Secondly, parameter estimation was performed locally on each reaction, which has now been updated to provide global parameter estimation for the system as a whole. Thirdly, the parameter estimation was skewed to favour flux values at steady state, which resulted in limited use for the models generated. In order to improve on this, the fitness measurement in the genetic algorithm used for parameter estimation has been updated to account for metabolite values as well flux and protein values.

After the model is built, it can be exported in SBML format to perform dynamic simulations and analysis using COPASI. As a proof of concept, a model of yeast glycolysis is being built using flux values, metabolite concentrations and protein amounts during steady state.

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

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Keywords: dynamic flux variability analysis, sugars, fatty liver, insulin signalling

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 objective of this work is to develop a predictive, multi-scale model of human hepatic monosaccharide transport, signalling and metabolism. This computational model will be used to 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), the aim of this work is to build a multi-scale model composed of: (i) gene regulation and signalling relevant to lipid and sugar metabolism; (ii) a kinetic model of insulin signalling created by integration of published ODE models; and (iii) the HepatoNet1 liver-specific genome-scale metabolic network constrained by in vitro flux measurements. In these simulations, we propose a novel analysis approach ‘dynamic flux variability analysis’ (dFVA). Here, the exchange flux of interest was set as the objective function and used the minimal and maximal objective function values to calculate upper and lower bound time courses of metabolite concentration in the medium or 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. Insulin sensitivity by western blot analysis (n=3-4) and sugar uptake with and without insulin stimulation (n=3-5) were measured.

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). Together with our newly proposed dFVA method, simulations have predicted minimum and maximum transport rates allowing the calculation of extracellular glucose and fructose concentrations over time. Insulin sensitivity was confirmed in HepG2 cells with a 1.7-fold increase of pAKT/AKT expression in response to postprandial levels of insulin. HepG2 medium glucose and fructose concentrations were found to be within our predicted dFVA solution space. In addition, a significant increase of sugar uptake was seen in insulin-treated versus untreated cells. 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. Future work will integrate the regulatory insulin signalling network to the metabolic network to predict the outcomes of insulin regulation on sugar and lipid metabolism in response to physiological levels of glucose and fructose in healthy and fatty liver.