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BOOK OF ABSTRACTS

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CONTENT

INVITED TALKS

From Atom to Adam - Evolving Cardiovascular Therapeutics Through Computational Modeling and Simulation

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One major challenge in understanding the effects of a drug on a complex organ such as the heart is to be able to simulate the various impacts of the drug all the way from the molecular level via intermediate pathways to the cellular level, and from there to the tissue and organ level where effects on heart function can be directly measured.
Computational modeling and simulation of a human heart requires including several physical fields traditionally treated as separate in the simulation world such as structural mechanics, fluid mechanics and electrodynamics. When coupled with systems biology and molecular modeling in a single scientific platform, the resulting integrated model can be used to better understand and predict the effects of drugs such as the appearance of life-threatening Torsade de Pointes arrhythmias.
In this presentation we will illustrate the way pharmaceutical researchers and their partners can work in a single scientific platform, through global scientific collaboration, scientific content federation and semantic search, and integrative modeling and simulation, to quickly and more effectively create new drugs and improve the success rate of delivering new, better targeted therapeutic solutions.

Signaling, state transitions, and geography of the normal and neoplastic colon

*Ken Lau** [1]
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The proliferation of high throughput single-cell technologies that can profile thousands of cells has led to an explosion of studies utilizing these techniques. However, analyses that move beyond cell type identification from these high-resolution data
are only emerging. Here, we explore quantitative, systems level tools that extract insight from multiplex single-cell data relating to pathways, transcriptional programs, and resulting cell-state transitions. We apply these tools to deconstruct the transitional programs of the normal colon and different colorectal cancer models from single-cell RNA-seq data. Our approach adds a layer of depth to the characterization of the plasticity of normal and neoplastic cells, and potentially to the understanding heterogeneous responses to perturbations.

Keywords: single-cell RNA-seq, trajectories, pseudotime

Computational methods and models for cellular systems biology

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Most cells possess the ability to change morphology or migrate in response to environmental cues. To understand the molecular mechanisms that drive cell movement requires a systems-level approach that combines computational approaches, including mathematical modeling and image analysis tools, with live cell microscopy. Here we present examples for how such an integrated research strategy has been successfully applied. First, we develop a stochastic model of cell migration and use it to investigate the role of the Rho GTPase RhoG in cell migration. Next mathematical modeling and quantitative image analysis methods are used to investigate the role of cerebral cavernous malformation (CCM) proteins in vascular tube formation.

Decoding cellular signals: irreversible commitment during hES cells differentiation

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During cell decision-making, gene and protein networks dynamically change in response to cues in order to trigger different cellular states. How information is decoded and transmitted in order to commit to specific cell fates has been a fundamental question in cell and developmental biology. One way to understand this is to monitor input-output relationships in single cells during decision-making.
In this context, my lab have been focused on investigating spatio-temp control and remodelling of intracellular networks in two important cellular decisions: cell division and cellular differentiation. Human embryonic stem cells (hESC) have the propensity to differentiate into the three germ layers (endoderm, mesoderm and ectoderm) and are therefore an ideal system to address how extracellular cues are decoded in order to commit to a specific cell fate. The switch between pluripotency and differentiation in these cells has been our paradigm of choice to understand how protein and gene

networks decode cellular signals and thereby encode commitment to different fates.
In my talk I will be presenting some of our unpublished work combining modelling and experiments to understand on how hESC irreversible commit to differentiation

Keywords: Fate decision, stem cells, signalling

Spatial omics of the mammalian liver

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The mammalian liver is composed of repeating anatomical units - the liver lobules, which are polarized by morphogens and blood flow. As a result of this graded microenvironment, hepatocytes and diverse non-parenchymal liver cells differ in their molecular signatures, depending on their lobule position. This phenomenon has been termed 'metabolic zonation'. I will describe spatial transcriptomics approaches that my lab has been applying to resolve the transcriptome-wide zonation signatures of all liver genes for both hepatocytes and liver endothelial cells. These approaches combine single molecule transcript imaging in the intact tissue, single cell RNA sequencing and paired-cell sequencing. I will also describe the use of 'spatial sorting' to interrogate the zonation of the liver proteome.

Systems Approaches to Metabolic Signaling

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The signalling network converging on phosphoinositide-3-kinases (PI3K) and mammalian/mechanistic target of rapamycin (mTOR) controls cellular growth and metabolism and is a central determinant of ageing and age-related diseases. mTOR responds to growth factors, nutrients, cellular energy, and a variety of stresses and controls virtually all anabolic processes including protein and nucleotide biosynthesis as well as lipid metabolism. While the core structure of the PI3K-mTOR network is well established, its wiring and dynamic behaviour differ depending on metabolic conditions as well as cell and tissue context. To systematically explore novel cues and connectivities across the PI3K-mTOR network, we combine detailed and omics wide computational and experimental analyses. Our current work elucidates the network structure in response to amino acids and stresses.

Network states in human metabolism

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Network-based analyses of 'omics' data are a cornerstone of systems medicine. The statistical task is to quantify the clustering of biological signals (e.g., significant expression changes) in a network (e.g., a metabolic network or a protein-interaction network).
Network coherences - topological indices evaluating the connectivity of subnetworks spanned by the 'omics' signal [1,2] - have been highly successful in identifying patient subgroups in disease cohorts [2-4].
Underlying this comparison of data with network architectures is the idea of considering the data as self-organized patterns on graphs.
In this talk I will briefly review this field for the case of human metabolic networks, starting from investigations of network architectures and then moving to dynamics on networks and, finally, to the concept of 'omics' data as patterns on networks and its application to data in biology and medicine.

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Keywords: Metabolic networks, pattern formation, 'omics' data

Mechanisms for and consequences of fatty liver disease

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Non-alcoholic fatty liver disease (NAFLD) is defined as hepatic fat accumulation that exceeds 5% of liver weight due to causes other than excessive alcohol use. In the past 2-3 decades, we have seen an unprecedented increase in NAFLD. Approximately 20-30% of adults in western countries have NAFLD, and its prevalence increases to 70-90% among adults with obesity or type 2 diabetes.
The underlying molecular mechanisms leading to the occurrence of NAFLD and its transition to severe liver disorders remain elusive, which limits the identification of drug targets and discovery of biomarkers that may be used to design effective treatment strategies.
To clarify the underlying metabolic disturbances in NAFLD, we investigated the metabolic differences in liver between subjects with varying degrees of HS by studying the kinetics of lipid metabolism. Using personalized genome-scale metabolic modelling, we elucidated an underlying molecular mechanism of NAFLD which can be used in the development of an effective treatment strategy.
Results indicated that plasma levels of glycine, serine, and associated metabolites were negatively correlated with liver fat content, suggesting that these GSH metabolism precursors might be limiting. To assess the effect of GSH and NAD+ repletion on the development of NAFLD, we added precursors for GSH and NAD+ biosynthesis to the Western diet and demonstrated that supplementation prevents HS in mice. In a proof-of-concept human study, we found improved liver function and decreased HS after supplementation with serine (a precursor to glycine) and hereby propose a strategy for NAFLD treatment.
We also performed a short-term intervention with an isocaloric low-carbohydrate diet in obese subjects with NAFLD and characterized the resulting alterations in metabolism and the gut microbiota using a multi-omics approach. We observed rapid and dramatic reductions of liver fat and other cardiometabolic risk factors paralleled by: marked decreases in hepatic de novo lipogenesis (DNL); large increases in serum beta-hydroxybutyrate concentrations, reflecting increased mitochondrial
beta-oxidation; and rapid increases in folate-producing Streptococcus and serum folate concentrations. Liver transcriptomic analysis on biopsy samples from a second cohort revealed downregulation of the fatty acid synthesis pathway and upregulation of folate-mediated one-carbon metabolism and fatty acid oxidation pathways.

Keywords: NAFLD, fatty liver, carbohydrates, NAD

Systems biology in hepatology: approaches and applications

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To develop novel strategies for prevention and treatment as well as to gain detailed insights about the underlying molecular mechanisms of liver associated diseases fatty liver disease, cirrhosis, type 2 diabetes and hepatocellular carcinoma, it is vital to study the biological functions of liver and how liver interacts with other human tissues as well as with the gut microbiota. Biological networks including metabolic, transcriptional regulatory, protein-protein interaction, signalling and co-expression networks can provide a scaffold for studying biological pathways operating in the liver in connection with disease development in a systematic manner. In my presentation, I will present our recent work where biological networks have been employed to identify the reprogramming in liver physiology in response to complex liver diseases. I will further discuss how this mechanistic modelling approach can contribute to the discovery of biomarkers and identification of drug targets which may lead to design of targeted and effective treatment strategies. Finally, I will present a roadmap for the successful integration of models of the liver and other human tissues with the gut microbiota to simulate whole-body metabolic functions in liver diseases.

In-Memory Data Management for Systems Biology

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Translating systems biology into clinical routine requires specific tool support, well-defined clinical process, and reproducible data processing. We share results of selected real-world research projects where we bring together experts from individual life sciences disciplines to design adequate software tools. Furthermore, we will share insight of our AnalyzeGenomes.com in-memory computing platform, which provides the technology foundation for interactive and reproducible life sciences research.

Liver multi-level hemodynamics and function simulations: towards a better understanding of surgery outcomes and disease progression

Irene Vignon-Clementel[1], *Chloé Audebert*[2], *Eric Vibert*[2]
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Authors: papers co-authors, N. Boissier and D. Drasdo (Inria & IFaDo), A. Daures and Ph. Rizzo (Fluoptics).

Liver is a key organ of the body, which function might be severely impaired due to disease progression or partial resection (pHx). Both trigger hemodynamics changes, which causes and consequences are still matter of debate. The precise link between liver architecture, perfusion and function remains to be fully elucidated.
First this work qualitatively and quantitatively characterizes the link between architecture and hemodynamics in the context of pHx [1], based on multi-level mathematical models of whole-body and hepatic hemodynamics [2]. The hepatic model takes into account a lobe-specific perfusion, with both arterial and portal venous inflows. Second we characterize such link at different stages of cirrhosis development [3]. For both different mechanisms impacting hemodynamics were studied (organ vascular dilation, liver micro-circulation changes, etc): systemic vascular responses seem particularly important to take into account to understand liver hemodynamics changes due to pHx, early days of regeneration and disease progression.
The third piece of the puzzle is function. ICG is an injectable compound, which is a marker of liver function. Its fluorescence dynamics can be interpreted via a dedicated pharmacokinetics model to provide perfusion and function information [4]. Such dynamic signal, recorded during surgery, is a novel way to characterize the liver state intraoperatively [5].
The above projects have been carried out based on animal experiments. The results, although encouraging, are the first steps, that need to be confirmed by on-going clinical translation.

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Whole-body/organ Imaging with Single-cell Resolution Toward Organism-level Systems Biology

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Organism-level systems biology aims to identify and analyze cellular circuits in organisms. To this end, one of the most powerful methods is optical imaging in combination with fluorescent labeling. However, the long-standing obstacle has been tissue opacity. Recently, the solutions to this problem have started to emerge by whole-body/organ clearing techniques that employ new tissue-clearing chemistry. In this talk, I introduce these advancements and discuss how to combine new clearing
techniques with efficient production of genome-engineered animals, rapid volume imaging and efficient image informatics in order to obtain quantitative organ-wide single-cell-resolution data. These technologies start to bring us closer to system-level understanding of physiology and diseases of complex mammalian systems.

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Keywords: single-cell-resolution

Integrative Approaches to Reconstruct Tumor Microenvironment Interactions

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The tumor microenvironment is a complex interaction of malignant and stromal cells. Recent technological advances in single-cell RNA sequencing and highly multiplexed in-situ imaging are providing a deeper characterization of the tumor microenvironment. In addition, new computational techniques that enable cell-specific deconvolution have been applied to bulk gene expression datasets to produce a pan-cancer landscape of the tumor microenvironment. While the composition of the tumor microenvironment is being better characterized, more progress still needs to be made to reconstruct functional interactions between cell types comprising the tumor microenvironment. I will present a variety of integrative computational approaches that combine imaging, genomic and proteomic data to infer cell-cell functional interactions that are critical to tumor maintenance and progression and promise to have therapeutic significance.

Multiscale mechanobiological models for developing asthma therapies

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Asthma is characterised by inflammation, airway hyper-responsiveness (rapid and excessive airway smooth muscle (ASM) contraction to low doses of contractile agent) and airway remodelling (involving long-term structural changes to the epithelium, collagenous basement membrane and ASM bundles). The mechanisms underlying these characteristics and how they interact is not well-understood. In this talk I will describe the multiscale models that we have developed, that capture ASM force generation cell-matrix adhesion in a dynamic environment at the cellular scale and its effect on tissue-level processes such as broncho-constriction/dilation of whole airways. Additionally I will illustrate how we are coupling these models to morphoelastic models of inflammation-driven airway remodelling, informed by experimental data, to understand the influence of remodelling on hyper-responsiveness and vice versa. These models have been developed in close collaboration with experimental biologists and respiratory clinicians with the ultimate aim of developing novel therapies driven by improved understanding of the underlying mechanobiology.

Keywords: airway hyper-responsiveness, airway remodelling, airway smooth muscle, bronchoconstriction, inflammation

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Synergism between computer modeling and cardiac imaging leads to improved understanding of right ventricular pump function

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We investigated how variations in physiology of the cardiovascular system can influence imaging-based measures, thereby identifying relevant interactions between cardiac components and properties. We used the multi-scale CircAdapt computational model to explore in silico the mechanics and hemodynamics of the adult heart and circulation in two specific clinical conditions: pulmonary arterial hypertension (PAH) and intense exercise.
Rapid leftward septal motion (RLSM) during early left ventricular (LV) diastole is observed in patients with PAH and its presence has been associated with the severity of this disease. Increased right ventricular (RV) wall tension induced by raised right ventricular afterload in PAH has been hypothesized to cause this abnormal motion. However, recent evidence has suggested that septal motion in PAH can also be reduced by onset of left-sided pathologies. Our modeling results showed prolonged RV shortening in PAH that caused interventricular relaxation dyssynchrony and RLSM. RLSM was observed in both moderate and severe PAH. A negative transseptal pressure gradient only occurred in severe PAH demonstrating that negative pressure gradient does not entirely explain septal motion abnormalities. PAH coexisting with RV contractile dysfunction exacerbated both interventricular relaxation dyssynchrony and RLSM. LV contractile dysfunction reduced both interventricular relaxation
dyssynchrony and RLSM. We conclude that onset of RLSM in patients with PAH appears to indicate a worsening in RV function and, hence, can be used as sign of RV failure. However, altered RLSM does not necessarily imply an altered RV afterload, but can also indicate altered interplay of RV and LV contractile function. Reduction of RLSM can result from either

improved RV function or a deterioration of LV function.
Exercise-induced RV dysfunction and disproportionate increases in pulmonary pressures have been associated with adverse RV remodelling. The CircAdapt computational model was used to simulate heart and circulatory dynamics during exercise. We thereby evaluated the effects of cardiac training and inotropy that can affect RV contractile function, as well as pericardial constraint that can modulate ventricular interaction. Our simulation results show that the exercise-induced increase in RV afterload led to impaired cardiac function and early-diastolic abnormal septal motion. Factors strengthening RV contractile function improved global cardiac function during intense exercise. The latter suggests that RV myocardial dysfunction limits cardiovascular exercise capacity.

Personalized modelling of bile acid metabolism: predicting metabolic effects of bariatric surgery

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Bile acids fulfill a variety of metabolic functions including regulation of glucose and lipid metabolism. Since changes of bile acid metabolism accompany obesity, Type 2 Diabetes and bariatric surgery, there is currently great interest in their role in metabolic health. We use a data-driven physiological modelling approach to obtain quantitative insight into the factors affecting bile acid metabolism. We developed a mathematical model that represents the enterohepatic and peripheral circulation of all major human bile acids. The model includes multiple time scales. It describes 24 hour dynamics associated with responses to meals. Slower dynamics mainly originate from processes in the colon and include transformation of bile acids by gut microbiota. The model is applied to analyse variability in bile acid metabolism in healthy individuals, to identify differences in patients with Type 2 Diabetes and predict metabolic effects of bariatric surgery. Identifiability of model parameters from plasma time-series metabolomics has been determined. A library of 'virtual patients' is created by calibrating the model to data from individuals who repeatedly received the same meal.
Models for 15 patients with Type 2 Diabetes are compared against an equal number of healthy controls, identifying differences in bile acid homeostasis and metabolic regulation. Next, the model is adopted to simulate both acute effects of bariatric surgery (within weeks after surgery) and slower physiological and metabolic adaptations that emerge in months and years after surgery. With the model we are able to test numerous factors that could contribute to changes in bile acids after Roux-en-Y gastric bypass in order to isolate the main contributors. The model demonstrates a strong influence of intestinal transit parameters on concentrations of plasma bile acids. In addition to immediate effects of the surgical intervention on intestinal transit, the observed slow progression is probably caused by self-regulating FXR feedback and/or adapted FXR feedback secondary to metabolic improvement.

Keywords: Systems Medicine, Metabolic Syndrome, Type 2 Diabetes, bariatric surgery, bile acids, metabolomics, virtual patients, data-driven physiological modelling

Systems Medicine Approaching Liver Surgery

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In the growing elderly societies, the incidence of primary and secondary liver tumors is continuously increasing. Surgical interventions like partial liver resections are often the only curative therapy removing more than 70% of the total liver mass. In humans, 20% to 30% of the remaining liver is required for liver regeneration. Successful restoration of the liver mass ultimately depends on the regenerative and metabolic capacity of the hepatocytes in the remnant liver. This, however, might be limited by individual factors like age, preceding chemotherapy, or by metabolic challenges like liver steatosis. The balance between
sufficient tissue removal to avoid tumor recurrence and maintaining a minimal functional liver mass for regenerative and metabolic homeostasis today depends on the surgeon's individual decision. Currently, liver surgery may be supported by computational tools, which take into account the location of the tumor relative to the liver vascular tree estimating volume and perfusion to predict post-surgery liver performance. This approach might misestimate the remnant liver function, because it does not consider functional aspects on the cellular level. These, however, are of ultimate importance to predict the regenerative and metabolic capacity of the remnant liver as a whole. E.g., post-resection lipid accumulation in the hepatocytes is necessary to provide energy substrates for liver regeneration. However, "metabolic overload" after extended resections is often the cause of post-surgery liver failure. Since lipid metabolism in the liver features both regional and zonal heterogeneity, integration of metabolic computational models on the cellular level would clearly support pre-surgical planning.
Current surgical planning tools focus on the estimation of liver volume as a surrogate predictor of remnant liver function. The underlying assumption is that all hepatocytes contribute equally to liver function. This, however,

neglects the spatial heterogeneity of liver metabolism and perfusion, potential alterations of hepatic function in the presence of a liver disease, or individual variations in metabolic function due to genetic variants, or as a consequence of lifestyle. A systems medicine approach including these biological, medical, and surgical aspects with available anatomical and functional information of the individual patient holds the promise for better prediction of postoperative liver function and hence improved risk assessment. Regulation and maintenance of liver function involves complex biological processes spanning multiple spatial and temp scales. Spatial scales range from the intracellular level up to the level of the organism, whereas temp scales have to reflect time periods of seconds to years (e.g., metabolism in seconds to days, regeneration over weeks, or disease progression over months). Thus, multi-scale-oriented modeling approaches are especially suited to provide a more comprehensive understanding of hepatic processes and mechanisms relevant in the context of hepatic resection. We will present computational models/modeling approaches for addressing liver functions, which might be essential for future multi-scale models supporting liver resection: (a) the hepatic stress response following physical damage, (b) the metabolic pathways affected by surgery, as well as (c) the regeneration of liver volume and function recovery. Our focus lies on selected liver-specific models from the field of systems biology relevant for liver surgery.

CONTRIBUTED TALKS

Disentangling the complex contribution of IL-6 signaling to Drug-Induced Liver Injury with dynamic pathway modeling

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Interleukin-6 (IL-6) is a key effector cytokine with a central role in liver regeneration and inflammatory processes. Recent findings also link IL-6 signaling to Drug-Induced Liver Injury (DILI), which is an adverse reaction in a small population of individuals upon administration of a drug. Diclofenac (DCF) and acetaminophen (APAP), both regularly prescribed
anti-inflammatory and analgesic drugs, are frequent causes of DILI, and the underlying molecular mechanisms are only partially understood. A systems biology approach is necessary to disentangle the complex contribution of IL-6 signaling to liver injury, induced by DCF and APAP.
By applying quantitative immunoblotting and qRT-PCR we first examined the dynamics of IL-6 pathway components in response to an addition of IL-6 alone or in combination with DCF or APAP in the human hepatocellular carcinoma cell line HepG2 and in cultured primary human hepatocytes. In comparison to IL-6 alone, the combined treatments prolonged STAT3 phosphorylation
and decreased the steady state level. While DCF drastically increased the amplitude in mRNA expression of pathway inhibitor SOCS3, APAP only delayed its peak and preserved the expression level. Both treatments also increased the mRNA expression of iron regulator Hepcidin, but DCF to a higher degree than APAP. Since these changes appeared rapidly after drug administration, they most likely directly arise from the parent compound.
To disentangle the complex effects of APAP and DCF on IL-6 signaling, we adapted our established dynamic pathway model of IL-6 signal transduction to the human context and integrated the acquired quantitative data. By applying L1 regularization on the drug induced changes in model reaction rates, we performed a structured analysis of possible points of interference of DCF and APAP in the molecular network. This analysis identified reactions, concerning the maturation and stability of SOCS3 mRNA and of the total SOCS3 levels as most affected. These drug-induced effects were experimentally verified and included into the mathematical model. However, the IL-6/STAT3 axis alone was not sufficient for the model to explain the drug effect on Hepcidin and in this way provided evidence that an additional input was needed. Based on experiments, the mathematical model was extended with a supplementary activation of the BMP/SMAD signaling axis by the drugs, with which it could finally fully explain the effects of DCF and APAP on IL-6-induced target gene expression.
Our approach shows how drug-cytokine interactions can effectively be characterized by the combination of quantitative data and dynamic pathway modeling. By further elucidating the molecular mechanisms underpinning the effect of DCF and APAP on inflammatory signaling in hepatocytes, an improved quantitative prediction of DILI and therefore a better risk assessment could be possible.

Keywords: IL-6, DILI, Diclofenac, Acetaminophen, Systems Biology

Mechanisms of reliable information transmission in JAK/STAT signalling

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Cellular communication is crucial for the homeostasis of multicellular organisms. Hum communication between cells is mediated by soluble factors that initiate intracellular signalling pathways. Notably, expression and activation of signalling proteins differs strongly even between isogenic cells of the same cell type. Obviously, this cell to cell variability does not prevent appropriate hum communication as complex molecular mechanisms evolved that guarantee reliable communication. The evolutionary conserved JAK/STAT signalling pathway is involved in regulation of the immune system, differentiation, growth, and regeneration. Dysregulated JAK/STAT signalling is associated with detrimental developmental, inflammatory, and neoplastic disorders. This study aims at clarifying molecular mechanisms, which facilitate reliable signal transmission in JAK/STAT signalling. We applied multiplexed single cell analyses and information theoretic measures to study robustness and Channel Capacity of
IL-6-induced JAK/STAT signalling. We show that factors limiting STAT3 tyrosine phosphorylation such as low concentrations of cytokine, negative feedback mechanisms, and STAT3 serine 727 phosphorylation enable robust activation of STAT3. Additionally, robustness can be ensured by increasing STAT3 copy number. Furthermore, Channel Capacity of IL-6-induced JAK/STAT signalling is reduced by cell-to-cell variability in STAT3 copy number, whereas

strong STAT3 activation and high STAT3 expression increase Channel Capacity. In summary, we elucidate molecular mechanism enabling reliable JAK/STAT signalling despite cell-to-cell heterogeneity, which contributes to preventing disease-associated dysregulated signalling.

Keywords: Information Theory, IL-6, JAK/STAT, Robustness

Predicting drug-induced metabolic perturbations with integrated whole-body PBPK & metabolic network models

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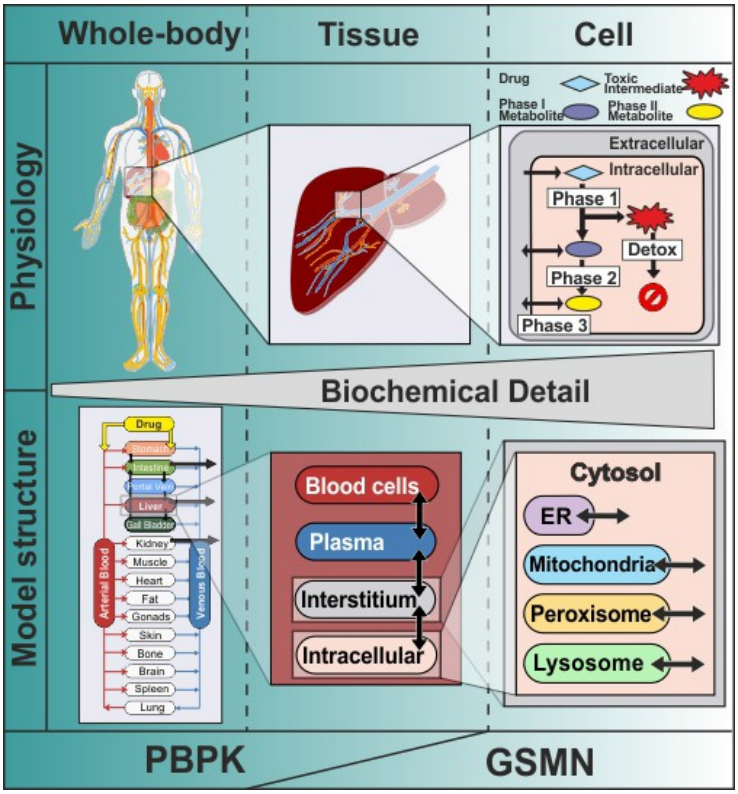
Objectives: We here present the computational multi-scale workflow that combines whole-body physiologically based pharmacokinetic (PBPK) models and organ-specific genome-scale metabolic network (GSMN) models through shared reactions of the xenobiotic metabolism. The applicability of the proposed workflow is illustrated for an antibacterial agent, which is known to cause drug-induced liver injuries.
Methods: Comprehensive phenotype-specific PBPK models of isoniazid and its metabolites were combined with GSMN models of the human liver. To this end, shared reactions of the xenobiotic metabolism of the PBPK model were introduced in the GSMN. The iterative application of the minimization of metabolic adjustment algorithm mapped the PBPK model derived pharmacokinetics on to the GSMN model and predicts minimal metabolic changes in the endogenous metabolism.



Integrating PBPK and GSMN models

Results: The combined PBPK-GSMN models quantitatively describe the whole-body pharmacokinetics of isoniazid and its metabolites and the intracellular metabolic responses in the liver. Intracellular and extracellular responses identified with the PBPK-GSMN models are in line with experimental and clinical findings. Moreover, the combined model shows how drug-induced metabolic perturbations are distributed and attenuated throughout the endogenous metabolic network. Further, combined PBPK-GSMN models predict the anticipated changes of blood metabolite pools.
Conclusions: Our simulation results show that a simultaneous consideration of both pharmacokinetics at the whole-body and metabolism at the cellular level is mandatory to understand drug-induced injuries at the patient level. The proposed workflow extends our mechanistic understanding of the biochemistry underlying adverse events and may be used to prevent drug-induced injuries in the future.

Keywords: PBPK modeling, Pharmacokinetics, Drug-induced metabolic perturbations, Metabolic networks, flux balance analysis, Computational systems biology, Systems toxicology

Deblurring spatio-temp signal network dynamics

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To steer dynamic changes in their structure and function, cells process information via signal networks in space and time. Acute optogenetic perturbation techniques are powerful tools to study these signal networks. However, diffusion of signal network components limits the spatial precision of perturbations, which blurs the analysis of spatio-temp signal processing. To sharpen our view on signal network dynamics, we recently developed "Molecular Activity Painting", a novel technology that is based on plasma membrane recruitment of signal molecules via photoactivatable chemically induced dimerization. Targeting to artificial receptors that are immobilized via surface-linked antibodies blocks lateral diffusion of recruited molecules. This enables rapid light-triggered "painting" of signaling molecules and their activity at the plasma membrane of living cells with micrometer precision that is induced within milliseconds and stable for hours. Using this method, we investigate spatio-temp processing in an excitable signal network that controls subcellular pulses, oscillations and waves of cell contraction. Based on our experimental investigations, we developed and analyzed a mathematical model for the spatio-temp self-organization of cell contraction dynamics that is based on reactions and diffusion of the small GTPase Rho and associated regulators and effectors. Predictions obtained from simulations of this model were confirmed experimentally. We propose that self-organized cell contraction dynamics enable an active, exploratory process that locally probes the elasticity of the extracellular environment.

Keywords: RhoA, Myosin, excitable, optogenetics, activity sensors, live cell imaging, microscopy, reaction-diffusion system, self-organization, emergence, oscillations, bifurcation analysis, ODE simulations, cellular automata

pyABC: A framework for distributed likelihood-free inference

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Mathematical models are widely used in computational biology to describe and analyze dynamic systems. The unknown model parameters or structures are often inferred using likelihood-based approaches. However, as models get more complex or stochastic, e.g. for multi-scale or agent-based models, the likelihood evaluation often becomes computationally intractable, while
it is still possible to simulate from models. To address this problem, Approximate Bayesian Computation (ABC) methods have been developed. In a nutshell, data are simulated for sampled parameters and compared to measured data via low-dimensional summary statistics, and accepted if sufficiently close according to some distance measure. These particles then realize a sample from an approximate posterior distribution. To tackle low acceptance rates and improve performance, ABC is frequently combined with a Sequential Monte Carlo (SMC) scheme, where the posterior approximation is iteratively improved throughout multiple particle populations. While ABC methods are broadly applicable, the computation time scales with the model simulations and is often limiting.
We developed pyABC, a distributed ABC-SMC framework for parameter estimation and model selection, which implements various state-of-the-art methods: To scale likelihood-free inference to computationally demanding models we implemented a
runtime-minimizing dynamic parallelization strategy for multi-core and distributed environments scaling to thousands of cores. Also, we developed a scheme for the automatic adaptation of population sizes, which in the vanilla approach is a sensitive tuning parameter. Further, we implemented a recently suggested strategy of adaptive weights to balance the impact of different summary statistics, which in particular avoids the need for pre-calibration and identifies non-informative statistics. The toolbox is easily accessible for non-expert users and offers multiple extension and customization possibilities to advanced users, such as acceptance threshold schedules, transition kernels, distance functions, early rejection, and data querying and visualization. Both the PYTHON and R languages are tightly supported. The source code is hosted on *https://github.com/icb-dcm/pyabc*.
pyABC has already been successfully applied in various application projects, e.g. to a study of tumor growth using a multi-scale hybrid discrete-continuum model, and to study virus spread using an agent-based model. The toolbox is already used by several international research groups and actively developed. This contribution will provide a comprehensive overview over the capabilities of pyABC and outline how many computational biology projects can benefit from it.

Keywords: computational biology, parameter estimation, model selection, approximate bayesian computation (ABC), stochastic models, multi-scale models

An intuitive and efficient approach for testing parameter identifiability

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The feasibility of uniquely estimating parameters of mathematical models from observations is a widely discussed aspect of systems biology. Several approaches have been published for analyzing this so-called "identifiability" of model parameters. However, they are typically computationally demanding, difficult to perform and/or not applicable in many application settings.
Here, an approach is presented which enables quickly testing of parameter identifiability. Numerical optimization with a penalty in radial direction enforcing displacement of the parameters is used to check whether estimated parameters are unique, or whether the parameters can be altered without loss of agreement with the data indicating non-identifiability. This so-called
"Identifiability-Test by Radial Penalization" (ITRP) can be employed for every model where optimization-based parameter estimation like least-squares or maximum likelihood is feasible and is therefore applicable for all typical models in systems biology. The approach is tested and briefly illustrated using 11 ordinary differential equation (ODE) models.
The presented approach can be implemented without great efforts in any modelling framework. It is available within the free Matlab-based modelling toolbox Data2Dynamics.
Source code is available at *https://github.com/Data2Dynamics*.

Keywords: Mathematical modelling, identifiability, parameter estimation, ODE models, nonlinear dynamics

Unveiling of conserved transcriptomics perturbation responses in mice and human

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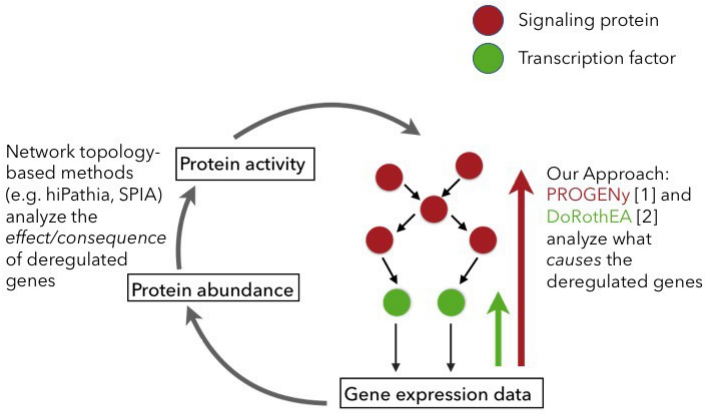
Functional analysis of transcriptomics data is an effective tool to acquire more mechanistic insights into human diseases, such as liver cirrhosis. Summarizing the large space of gene expression values into smaller number of biological meaningful features (e.g. pathway activities) not only helps to reduce experimental noise but can also help to identify the underlying mechanisms, and thereby propose novel therapies. However, the study of human diseases is often limited by the availability of patient data and ethical concerns. Therefore, these investigations often go along with the use of model organisms, commonly mice, especially if the study objective is assumed to be evolutionary conserved. Mouse and human share a highly conserved gene regulatory system, which suggests that gene regulation and signal transduction methods, developed originally for human applications, can also be applied directly on mice data.
We demonstrate this hypothesis using the tools PROGENy and DoRothEA, both data-driven methods recently developed in our group for human applications (see figure). PROGENy infers pathway activity from gene expression data. In comparison to conventional pathway analysis methods that use the genes of the pathway members, PROGENy calculates pathway activity based on consensus gene signatures obtained from perturbation experiments, outperforming conventional methods. DoRothEA is a framework to estimate the activity of transcription factors (TF) by applying enrichment analysis methods, where manually curated TF regulons serve as underlying gene sets.
We benchmarked the performance of both tools on publicly available human and mice single-gene and single-drug perturbation data. The benchmarking clearly shows that both tools can be applied to mouse data, with the corresponding area under the ROC curve comparable between mouse and human. In the case of DoRothEA it could be shown that the high-quality, manually curated human regulons applied on translated mice data even outperforms dedicated mouse TF regulons. From these results it can be concluded that the underlying response mechanisms triggered from perturbation experiments are highly conserved between mice and human, at least at the (approximate) level of current functional genomics tools. We are currently applying these methods to chronic liver disease data in the context of the LiSyM network.



[1] Schubert et al., *Nature Comm*, 2018 (Tool page: https://saezlab.github.io/progeny/)
[2] Garcia-Alsono et al., *Cancer Research*, 2017 (Tool page: https://saezlab.github.io/DoRothEA/)

Keywords: functional genomics, transcriptomics, gene signatures, perturbation signatures

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Characterization of NAFLD progression in Human Liver Biopsies by Shotgun Lipidomics

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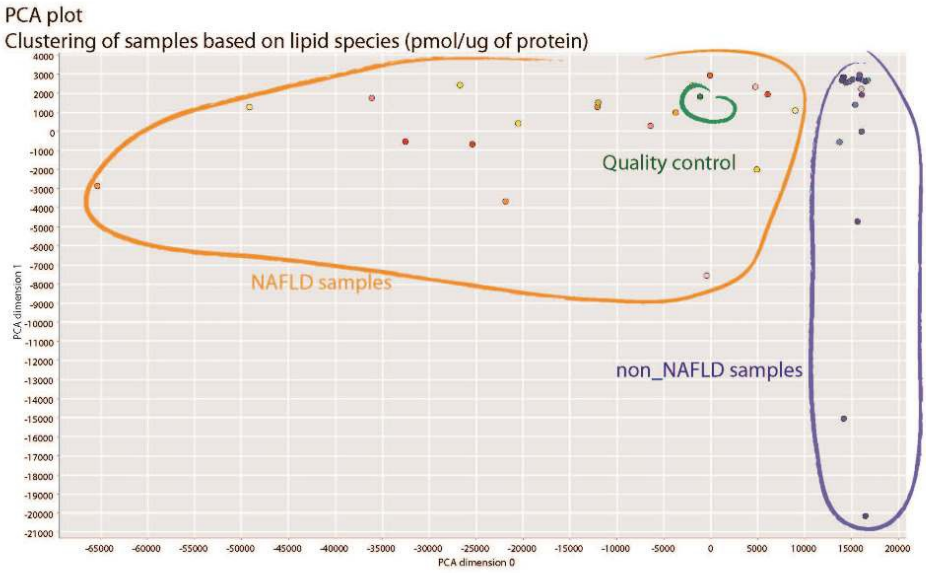
Non-alcoholic fatty liver disease (NAFLD) affects 10 to 35% of the population around the globe and manifests itself as the accumulation of neutral lipid in hepatocytes. If gone untreated, NAFLD may lead to the development of cirrhosis or liver cancer. We applied quantitative shotgun profiling to analyse the full lipidome of liver biopsies from patients screened for known NAFLD risk markers such as PNPL3. The 367 samples span a wide range of conditions such as hepatitis statuses, BMI, ages and disease states, including samples with liver cancer.
Preliminary analysis was performed on a pilot cohort consisting of 38 patients: 19 NAFLD patients and 19 control (non-NAFLD) patients (age 50+/-17, 9 males, 27 females). We quantified absolute (molar) content of 18 lipid classes covering 264 lipid species. A PCA plot built based on pmol of lipid species per ug of protein in the liver tissue (figure 1) revealed the separation of samples from different groups, where NAFLD samples cluster separately from non-NAFLD group.



Clustering of samples from pilot cohort based on pmol of lipid specie per ug of protein. Samples from NAFLD group (red and yellow) cluster separately from non-NAFLD group (blue and violet). Green dot indicates the quality control run.

Expectantly liver of NAFLD patients was enriched in neutral lipids. In particular, the amount of both triacylglycerols and diacylglycerols were elevated by 3-fold. At the same time, cholesteryl esters levels were ca. 2.5 times higher and

free cholesterol 1.5 times higher in fatty liver than in healthy control. Interestingly, in non-NAFLD samples the amounts of several phospholipids, such as PS and PC, as well as lysoPC, were increased by 1.5 to 2 times.
We are currently applying statistical analyses, such as boosted random forests and naïve bayes machine learning algorithms, to discover a network of biomarkers that would allow the early detection and differential diagnosis of NAFLD, cirrhosis or liver cancer.

MR elastography for assessing hepatic fibrosis and steatosis in pediatric non-alcoholic fatty liver disease

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Background: Non-alcoholic fatty liver disease (NAFLD) is the most frequent chronic liver disease in children today. Despite its invasiveness, liver biopsy is still the gold standard for the diagnosis of NAFLD. Magnetic resonance elastography (MRE) is a noninvasive imaging technique (1) used to probe the biomechanical properties of soft tissues in vivo and it has demonstrated great value in diagnosing liver fibrosis.
Methods: 50 patients (age range 1-17 years, 10 females) who are overweight or obese (average BMI: 33.9 kg/m2) and exhibit prolonged elevation of serum alanine aminotransferase (ALT) and/or aspartate aminotransferase (AST) (>50 U/l for at least 3 months) were recruited. MMRE (1) was conducted at 1.5 T scanner (Siemens, Magnetom Sonata) using 7 harmonic frequencies (30 to 60 Hz, 5 Hz increment), the abdominal MRE setup is the same as described in (2). The 3D wave field was recorded using a single-shot EPI sequence with motion-encoding gradients (MEG). Total acquisition time for 9 consecutive slices of 2.7 × 2.7 × 5 mm3 resolution, 7 frequencies, 8 wave dynamics was 5 minutes and 8 seconds. MRE wave data was reconstructed using wavenumber based inversion method as detailed in (3), yielding parameter map of the shear wave speed (c) which represents mainly the liver stiffness and penetration rate (a) which reflect the tissue damping. Hepatic fat fraction (HFF) was also estimated with Dixon method. Liver biopsy was performed for fibrosis and steatosis grading.
Results: Based on histological staging, 28 subjects had no or early fibrosis, 15 subjects with stage 0 (F0), 12 with stage 1 (F1). 9 subjects had stage 2 (F2) moderate fibrosis and 14 subjects had advanced fibrosis with stage 3 (F3). Based on the steatosis grade, the patients are divided into 3 groups, with 10, 17 and 23 subjects having steatosis grade 1, 2 and 3 (S1,S2 and S3), respectively. In term of fibrosis, c was significantly higher in patients with F3. c was also used for detecting any fibrosis (F 1), moderate
fibrosis (F 2) and advanced fibrosis (F 3) with AUROC of 0.79, 0.91 and 0.90, correspondingly. Regarding steatosis, a was significantly lower in S3 group while HFF was elevated with increasing steatosis grades. Both a and HFF were used to detect moderate steatosis (S 2). The AUROC for a and HFF are 0.86 and 0.92, respectively. Additionally, a negative correlation between a and HFF (Person r = -0.6, $P < 0.0001$) was obtained.
Conclusions: Wave speed obtained from MRE is sensitive in differentiating moderate and advanced fibrosis. Penetration rate as another MRE parameter is able to detect moderate steatosis and is negatively correlated with HFF. Both MRE derived mechanical parameters c and a are independently responsive to pathological feature of NAFLD such as fibrosis and steatosis. The mechanical parameters can potentially serve as a complementary imaging marker for the noninvasive assessment of liver fibrosis and steatosis in patients with NAFLD.

Keywords: magnetic resonance elastography, non-alcoholic fatty liver disease, liver stiffness

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In Silico Endovascular Repair of Patient-Specific Abdominal Aortic Aneurysms

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Endovascular aneurysm repair (EVAR) is a widely used and well established technique to intervene before rupture of abdominal aortic aneurysms (AAA) occurs. However, EVAR can involve some unfavorable complications such as endoleaks or stent-graft (SG) migration. Such complications, resulting from the complex mechanical interaction of vascular tissue, SG and blood flow or incompatibility of SG design and vessel geometry, are difficult to predict. Computational vascular mechanics models can be a predictive tool for the selection, sizing and placement process of SGs depending on the patient-specific vessel geometry and hence reduce the risk of potential complications after EVAR [1]. In this contribution, we present a new in silico EVAR methodology to predict the final state of the deployed SG after intervention and evaluate the mechanical state of vessel and SG, such as contact forces or wall stresses. Four different constituents of the vascular tissue are considered: healthy vessel wall, diseased aneurysmatic wall, intraluminal thrombus and calcifications [2]. We consider mortar based frictional contact [3] between a sophisticated AAA model and a SG composed of a parameterized, product specific graft shell and stent wire frame that can undergo finite deformations. The simulation results of three patient-specific cases are compared to the geometry of the deployed SG taken from

postinterventional CT scans and the quality of the predictive capability is quantified. Further, we suggest a parameter continuation approach [4] to model various different sizes of SGs within one in silico EVAR simulation which can be a valuable tool when investigating the issue of SG oversizing.

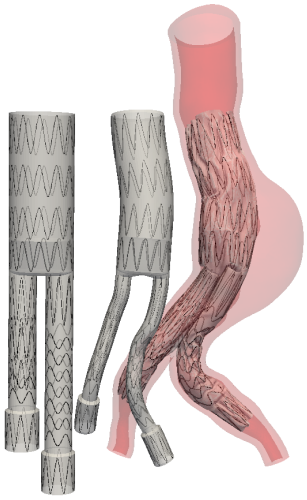

Figure 1: In silico stent-graft placement and deployment.

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Keywords: Abdominal aortic aneurysm, Stent-graft, Computational vascular mechanics

POSTERS

Signal Transduction & Inflammation

SBMC18-57

Prediction of pathway desensitization by mathematical modeling of interferon alpha signal transduction

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Cellular signaling cascades adapt to changing environments through multiple feedback mechanisms. Pre-activation of the interferon alpha (IFNα) signal-transduction pathway can cause a desensitized state in which the system is not responding to further treatment which constitutes a serious therapeutic problem. Even though the involvement of USP18 as one of the key negative regulators has been reported, the underlying mechanisms as well as the role of further feedback components remain unresolved.
Calibrated by quantitative time-resolved measurements, we here present an ordinary differential equation model for IFNα signaling that comprises seven different feedback regulators (STAT1, STAT2, IRF9, USP18, SOCS1, SOCS3 and IRF2) and covers a time-span of 32 hours including multiple treatments. We show that pre-stimulation with a high dose of IFNα leads to a gradual dose-dependent desensitization, while a low dose of IFNα leads to a hypersensitization of the pathway. By means of measurements with a stable USP18-inducible cell line, we further show that USP18 alone is insufficient to desensitize the pathway. By hypotheses testing of different model structures, we reveal SOCS1 as a relevant co-factor for USP18. In our model, presence of USP18 inhibits the activation of the receptor and on top enhances receptor degradation that is mediated by SOCS1. While desensitization is established at the receptor level, hypersensitization is created by the induction of the signaling proteins STAT2 and IRF9.
Model simulations show that the levels of STAT2 and USP18 are the most relevant components to determine the dose-dependent hypersensitization and desensitization of the system. Interestingly, although SOCS1 is involved in receptor degradation, the level of the receptor after stimulation is mainly dependent on the level of USP18. By the determination of molecules per cell, we revealed patient-to-patient variability of the abundance of USP18 in primary human hepatocytes from patients and propose USP18 as predictor for patient-specific desensitization of the IFNα signaling pathway.

Keywords: IFN alpha signal transduction, Desensitization, USP18, Mathematical modeling

SBMC18-94

Acute liver failure and Acute-on-chronic liver failure: Impact of paracetamol on HGF-induced cell cycle progression

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The liver is the central metabolic organ and maintains the homeostasis of the organism as well as detoxification of xenobiotics, potentially leading to drug-induced liver injury (DILI) that can progress to acute liver failure (ALF). Chronic liver damage (CLD) leads to higher susceptibility of patients to suffer from acute-on-chronic liver failure (ACLF). On the other hand the liver has a remarkable capacity to regenerate to counteract tissue loss, a process that is coordinated by inflammatory cytokines and the hepatocyte growth factor (HGF).
To determine the impact of paracetamol (APAP, acetaminophen), a possible trigger for ACLF, on HGF-induced hepatocyte proliferation, we analysed HGF-induced cell cycle progression in hepatocytes isolated from transgenic mice harbouring a fluorescence ubiquitin cell cycle indicator (Fucci2). Hepatocytes were stimulated with HGF or HGF in combination with different APAP concentrations and were analysed by life cell imaging at the cell population as well as the single cell level. As expected, APAP showed a dose-dependent hepatoxic effect. However, single cell analysis revealed an APAP-mediated inhibition of
HGF-induced cell cycle progression in the S/G2-phase already at non-toxic concentrations of APAP. To address the impact of CLD, we fed mice harbouring the Fucci2 system for up to 26 weeks with western diet (WD) or standard diet (SD). Time-lapse microscopy of primary mouse hepatocytes isolated from these mice showed that hepatocytes from WD mice displayed a higher sensitivity towards APAP. Furthermore, we utilized a time-resolved mass spectrometric approach to identify key proteins that are affected by APAP and are altered upon CLD. This data together with our single cell studies is used to calibrate a dynamic pathway model to link HGF-induced signal transduction to the cell cycle progression and unravel molecular mechanisms of APAP-mediated cell cycle inhibition in ALF and ACLF.

Keywords: ALF; CLD; ACLF; DILI; APAP; cell cycle; HGF

SBMC18-38

Epigenomic map of human liver reveals principles of zonated morphogenic and metabolic control

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A deeper epigenomic understanding of spatial and functional organization of cells in human tissues is an important challenge. Here we report the first combined positional analysis of transcriptomes and methylomes across three micro-dissected zones (pericentral, intermediate and periportal) of human liver. We identify pronounced anti-correlated transcriptional and methylation gradients including a core of 271 genes controlling zonated metabolic and morphogen networks. A prominent
porto-central gradient of DNA methylation at binding sites for 46 ENCODE transcription factors points towards a methylation gradient dependent control of transcription. The gradient includes specific epigenetic and transcriptional signatures in the wnt pathway supporting the concept of a pericentral hepatocyte regeneration pathway in humans under steady-state conditions. Surprisingly, while metabolic gene expression changes across zones are observed with increasing severity of non-alcoholic fatty liver disease, the relative zonated expression and methylation differences across zones remain unchanged. This observation suggests a maintained molecular zonation during reversible early NASH states. Overall our data provide a wealth of new positional insights into zonal networks controlled by epigenetic and transcriptional gradients in the human liver lobule.

SBMC18-95

Dynamic pathway modeling of TGFbeta signaling in hepatic stellate cells

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Chronic Liver Disease (CLD) is a pathological process of consecutive destruction and regeneration of liver parenchyma that leads to liver fibrosis and cirrhosis. The major contributors of progressive liver fibrosis are hepatic stellate cells (HSC), which are activated by transforming growth factor beta (TGFbeta). Correspondingly, the TGFbeta signaling pathway emerged as a prospective target in the treatment of liver fibrosis. However, the outcome for such treatments is difficult to predict due to the absence of specific insights into the TGFbeta-induced signaling pathway in HSC during CLD progression.
To create a dynamic pathway model of the TGFbeta-induced Smad signaling pathway in HSCs, we utilized our dynamic pathway model of TGFbeta-signaling previously established for the hepatocellular carcinoma cell lines Hepa1-6 and HepG2, as well as for primary murine hepatocytes (Lucarelli et al., 2018). HSC lines JS1 (murine) and LX2 (human) were used as the representatives of murine and human HSCs. The abundance of Smad molecules per cell was different for the examined cell lines: Smad2 and Smad4 were predominantly present in Hepa1-6 and HepG2, whereas Smad2 and Smad3 prevailed in the HSC lines JS1 and LX2. Interestingly, the results of the time resolved experiments employing quantitative immunoblotting revealed a comparable dynamics of TGFbeta induced Smad2/3 phosphorylation in all cell lines. The mathematical model adapted to the cell-context specific abundances predicted that the difference of the Smad ratios affects the formation of Smad complexes. The complexes with Smad4 are primarily present in Hepa1-6 and HepG2 but poorly represented in the HSC cell lines JS1 and LX2. These model predictions were confirmed by time-resolved co-immunoprecipitation experiments.
To extend the model to the regulatory events occurring at the receptor level, we established a targeted-mass spectrometry approach for the TGFbeta receptor I (TGFBRI). The results of the receptor measurements demonstrate lower abundance of TGFBRI in HSC, whereas in hepatocellular carcinoma and primary murine hepatocytes the total number of TGFBRI is comparable.
In summary, the results demonstrate a difference in the abundance of different Smad complexes and type I receptor in HSC compared to hepatocellular carcinoma cell lines, which in turn can explain a different phenotypical response to the same TGFbeta stimuli. In perspective, the experimentally calibrated model will provide a better understanding of

CLD pathogenesis to refine a TGFbeta directed treatment approach for a safer clinical translation to patients with liver fibrosis.

Keywords: TGFbeta, Liver, Hepatic Stellate Cells, Smad complexes

SBMC18-64

Mesenchymal stromal cells improve multi-organ integrity after extended liver resection by targeting thrombospondin 1

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Extended liver resections carry a high risk of post-surgery multi-organ dysfunction. Amongst others, this is due to the development of hyperdynamic circulation associated with the hepatorenal syndrome, similarly as in acute-on-chronic liver failure. In a pig model of 70 % liver resection, mesenchymal stromal cells (MSC) have been shown to improve liver function after extended resection, which was associated with amelioration of circulatory hemodynamics. This suggested a systemic mode of
MSC action rather than a local impact on the liver. In order to identify systemic, organ-independent mechanisms of MSC action, we analyzed transcriptional changes in two different organs (liver, lung) 24 h after resection in animals with and without MSC treatment. Based on a genome-wide protein-protein interaction network analysis and the gene expression data, joint regulatory modules were identified, which by subsequent network inference revealed a possible role for thrombospondin-1 (THBS-1) in mediating the MSC effects. THBS-1 is a potent activator of latent TGF-beta, a major mediator of epithelial-mesenchymal
transitions during liver regeneration, of which, however, excessive actions might also contribute to organ damage. In line, extended liver resection increased serum TGF-beta and TGF-beta signaling accompanied by an increase of epithelial damage in liver and lungs. These were attenuated by MSC treatment. In cultures of primary porcine hepatocytes, MSC did not inhibit
TGF-beta signaling suggesting that they did not directly interfere with TGF-beta. This was corroborated using co-cultures of MSC with MDCK1 or Hep3B hepatoma cell lines. Thus, it is concluded that MSC indirectly inhibited TGF-beta signaling by decreasing THBS-1-mediated activation. It is highly likely that this is achieved by decreasing THBS-1 secretion by endothelial cells, the major source of circulatory THBS-1. It is our current hypothesis that the hyperdynamic circulation as provoked by extended liver resection imposes mechanical stress on vascular endothelial cells, thus stimulating THBS-1 secretion and downstream elevation of active TGF-beta. The hemodynamic improvement by MSC might attenuate circulatory stress and THBS-1 secretion, thereby preventing overwhelming provision of active TGF-beta in conjunction with epithelial damage in multiple organs.

Keywords: liver resection, stem cell therapy, thrombospondin-1, TGF-beta, pig

SBMC18-30

Liver sinusoidal endothelial cells trigger CD8 T cell mediated liver failure and herald liver regeneration

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Liver sinusoidal endothelial cells (LSECs) have unique immune functions and coordinate local immune responses in the liver. We have recently reported that LSECs are key for induction of T cell mediated liver failure by activation of CD8 T cells through cross-presentation. In LiSyM we have characterized the ability of LSECs to orchestrate also liver regeneration. Molecular correlates of hepatocyte death such as extracellular ATP, cell damage such as HMGB1, contact with apoptotic or necrotic hepatocyte material, or exposure to Toll like receptor ligands all failed to induce significant expression and release of HGF. This excluded a role of LSECs as sentinels of cell death in the chronically damaged liver that could act as rheostat to provide regenerative signals. We confirmed these in vitro findings in the LiSyM ring trial of metabolic liver damage, where we did not find evidence for increased activation of LSECs by immunohistochemistry of liver slices.
Rather we found that LSECs are potent producers of HGF in response to IL-6 cluster-signaling in a dose-dependent fashion. Since IL-6 cluster signaling is involved in co-stimulation of LSEC cross-presenting antigen to CD8 T cells, it is very likely that cognate interactions between LSECs and CD8 T cells involving IL-6 cluster-signaling trigger both, effector function of CD8 T cells killing hepatocytes as well as HGF release from LSECs. The dynamics of such IL-6

cluster-signaling and HGF production in the context of T cell induced liver damage is likely to provide crucial information to rescue the liver from failure, and is currently under investigation.
In summary, LSECs apparently orchestrate both, T cell-induced liver damage and liver regeneration, thereby acting as hepatic rheostats for organ homeostasis. Since IL-6 cluster-signaling simultaneously induces T cell activation and HGF production, we assume that liver regeneration is triggered at the time of induction of liver damage, which may explain the proverbial regenerative response of the liver to (immune-mediated) damage. While IL-6 cluster-signaling induced HGF-production by LSECs may contribute to liver regeneration in situations of T cell-mediated liver damage, liver regeneration in response to toxic agents without induction of IL-6 cluster-signaling or in the context of LSEC damage likely requires different cellular sources of HGF. Replacement of LSECs by macrovascular endothelial cells during severe chronic liver damage may therefore lead to deficient regenerative signals and pave the way for acute-on-chronic liver failure.

SBMC18-92

Receptor turnover and transcriptional negative feedbacks control cell type-specific dynamics of the TGFbeta pathway in lung cancer

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Rationale: Non-small-cell lung cancer (NSCLC) is the leading cause of cancer-related mortalities worldwide. Elevated levels of the Transforming Growth Factor-beta (TGFbeta) ligand correlate with a poor survival of lung cancer patients. However, the regulatory mechanisms controlling pathway activation in lung cancer remain poorly understood.
Aim: In the presented work we combined quantitative data generation and mechanistic mathematical modeling to understand how the dynamic properties of TGFbeta signal transduction are controlled.
Methods: The dynamics of TGFbeta-induced Smad2/3 phosphorylation was measured by quantitative immunoblotting in three NSCLC cell lines H1975, H838 and H1650. The levels of TGFbeta-receptor type I and type II were determined by a targeted mass spectrometry approach. Inhibitors of mRNA transcription, protein translation or protein degradation were used to perturb the signaling network. The expression of transcriptional negative feedback regulators was assessed by qRT-PCR. siRNA-mediated knockdowns were performed to examine the importance of different feedback regulators.
Results: The examined NSCLC cell lines expressed different amounts of the TGFbeta-receptors, showed a distinct dynamic behavior of the TGFbeta-induced Smad2/3 phosphorylation and differentially responded to inhibitor perturbations. These results suggested a differential prevalence of negative feedback regulators that induce the degradation of the TGFbeta-receptor or reduce its ability to phosphorylate Smads. The model-based analysis predicted that the unstable receptor is constantly degraded and produced again from stable mRNA. We experimentally confirmed a high stability of the TGFbeta-receptor mRNA, while the accumulation of the TGFbeta receptor protein upon inhibition of the proteasome function was validated by our targeted quantitative mass spectrometry approach.
Conclusion: These findings highlight that the TGFbeta receptor is one of the most sensitive nodes that controls pathway activation. Therefore, targeting in lung cancer processes that control receptor abundance rather than using conventional TGFbeta kinase inhibitors could be a promising therapeutic approach.

Keywords: TGFbeta, signal transduction, mathematical modeling, lung cancer

SBMC18-100

Multi-level mathematical model to simulate the early phase of pneumococcal alveolar infection

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Pneumonia is one of the most prevalent diseases worldwide, mainly caused by Streptococcus pneumoniae infection. There are parts of the population that present higher risk of pneumonia, namely infants, elderly and immunosuppressed people. These groups are more vulnerable because they have immature or impaired immune systems. The optimal therapeutic strategy for these groups would be preventing the disease, in order to decrease the irreversible damage form lungs inflammation. However pneumococci vaccines are less effective in these groups for the same reason they are more vulnerable. For this reason these groups of people would require specific strategies to prevent pneumococcal infection of the alveolar tissue.
We have proposed a computational approach to simulate the very first stages of the pneumococcal infection happening in a single alveolus. This biological condition represents the critical phase that should be controlled, but it becomes practically inaccessible

by in vivo experiments. We created a multi-level mathematical model that combines the intracellular signalling pathways and the cellular interactions that play a role during the early stages of pneumococcal alveolar infection. By systematic perturbations of the model we obtained a high number of solutions that we analysed by regression techniques. The results of the analysis of the simulations predict two main processes that could control the establishment of the infection in the alveolus. Bacterial proliferation and bacterial adherence together can predict the evolution of the early phases of the pneumococcal infection. We propose that controlling the pneumococcal adherence could be a potential strategy to prevent pneumonia in sensitive groups.

Keywords: multi-level modelling, pneumonia, streptococcus pneumoniae, inflammation

SBMC18-24

The complex life of Smad isoforms determines gene expression

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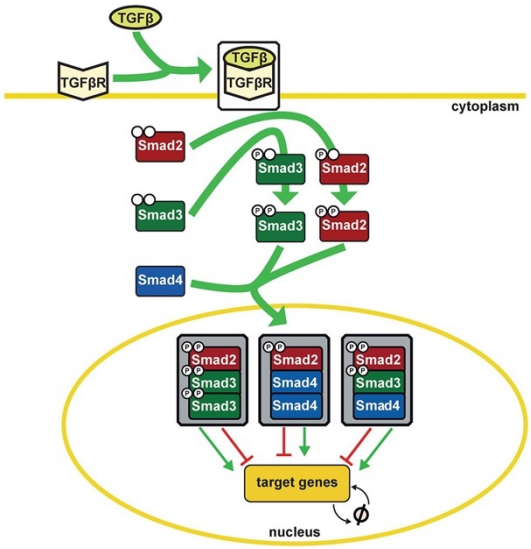
Transforming growth factor β (TGFβ) leads to the phosphorylation of the Smad isoforms Smad2 and Smad3. Doubly phosphorylated Smad proteins (ppSmad2 and ppSmad3) together with Smad4 can form different trimeric Smad complexes. The Smad complexes in turn activate a broad spectrum of target genes. It remains unresolved which of the possible Smad complexes are formed in cellular contexts and how these contribute to gene expression. We combine time- and dose-resolved experimental data obtained by quantitative immunoblotting and site-specific mass spectrometry with a computational selection strategy by L1 regularization. Our approach enabled us to predict and provide experimental evidence for the three most relevant Smad complexes in the mouse hepatoma cell line Hepa1-6: ppSmad2:ppSmad3:ppSmad3, ppSmad2:Smad4:Smad4, and ppSmad2:ppSmad3:Smad4. Utilizing dynamic pathway modeling, we specify the contribution of each of these Smad complexes to the expression of representative Smad target genes, and show that these contributions are conserved in human hepatoma cell lines and primary hepatocytes. We predict based on gene expression data of patient samples increased amounts of Smad2/3/4 proteins and Smad2 phosphorylation as hallmarks of hepatocellular carcinoma and experimentally verify this prediction. Our findings demonstrate that modeling approaches can disentangle the complexity of transcription factor complex formation and its impact on gene expression.



Reference:
Lucarelli et al. 2018, Resolving the Combinatorial Complexity of Smad Protein Complex Formation and Its Link to Gene Expression. Cell Systems 6(1):75-89.e11.

Keywords: L1 regularization; Smad proteins and complexes; TGF-beta signal transduction; dynamic pathway modeling; hepatocellular carcinoma; liver; mathematical modeling; quantitative mass spectrometry; regulation of gene expression; systems biology

SBMC18-69

Morphogens in the adult liver - a panlobular insight

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Morphogens like Hedgehog (Hh) and Wnt/beta-Catenin (Wnt) are known to govern developmental processes in embryogenesis, tissue differentiation and regeneration. More recently the impact of those morphogens on adult cell physiology and metabolism has sparked increasing interest. Most metabolic pathways in the liver are strongly zonated, partially caused by adapting to changing contents of nutrients and oxygen within the porto-central axis. How morphogens contribute to the lobular distribution of metabolic pathways is part of an evolving field in science since the last decade. For long, the pericentral located Wnt pathway was thought to be exclusively responsible for liver zonation of ammonia and glucose metabolism. Recently our group could demonstrate a dramatic impact of the Hh pathway on lipid metabolism in the adult liver.
For a better understanding of metabolic liver zonation under morphogenic control we aimed at demonstrating the mutual impact and zonal distribution of Hh and Wnt/beta-Catenin signaling in the adult liver of mice and human.
To investigate the impact of Hh- and Wnt-signaling on liver zonation different mouse models were bred which allow a hepatocyte-specific modulation of these pathways in adult mice. In addition human liver material was used. To depict the porto-central distribution of several pathway markers periportal (pp) and pericentral (pc) hepatocytes were isolated using digitonin-calagenase perfusion, followed by qPCR and metabolome analysis of both hepatocyte populations. Moreover protein distribution on IHC stained liver slices were quantified via the modular software tool TiQuant. In addition a proteomic and metabolomic analysis from isolated pp and pc hepatocytes, as well as liver material was performed allowing a global overview about the impact on metabolic functions of the liver by morphogens.
Our results indicate a strong relation between Wnt and Hh in the adult liver for the first time. The immunohistological analysis indicates how central proteins of Hh and Wnt are localized and how they influence each other when the pathways are modulated. Furthermore the outcome of the proteomic and metabolomic approach give a deep insight how morphogens regulate liver metabolism in detail and contribute to a better understanding of the fine tuning mechanism on metabolic liver zonation and may help to shed light on the underlying mechanisms of deregulations like NAFLD/NASH and systemic impairments like hepatic encephalopathy.

SBMC18-26

Estimating time delays using profile likelihood

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Time delays are present in many biological signaling pathways. The usual way of modeling these is via utilizing delay differential equations (DDEs) which require specialized numerical solvers. A much more practical way of dealing with delays is to introduce a set of intermediate non-observable states which delay signal propagation and produce a low-passed version of the input signal in contrast to the 'hard' delay obtained by using DDEs. For an appropriate choice of reaction rates this method, which is known as the linear chain trick, provides a good approximation to DDEs.
Biological systems rarely exhibit 'hard' delays whereas intermediate states often have an actual biological interpretation. In this work, we would like to determine to what extent the number of intermediate states can be inferred from data. Starting from statistical considerations one can derive a criterion to determine the optimal number of states by employing parameter identifiability analysis based on the profile likelihood which shows to be much more reliable than the analysis of best fits only.

Keywords: Time delay, parameter identifiability, profile likelihood, linear chain trick, signaling pathway, dynamical modeling

SBMC18-23

Mathematical modeling of drug-induced receptor internalization in breast cancer cells

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About 20% of breast cancer tumors over-express the HER2 receptor. Trastuzumab, an approved drug to treat this type of breast cancer, is an antibody directly binding at the HER2 receptor and inhibiting cell growth.
The goal of our study was to understand the early impact of trastuzumab on HER2 internalization and recycling in the HER2-positive SKBR3 cell line. To this end, single cell fluorescence microscopy, monitoring the state of HER2 expression on the membrane, was combined with mathematical modeling to derive the flux of HER2 receptors from and to the membrane. We constructed a dynamic multi-compartment model based on ordinary differential equations to account for intracellular HER2 production and distribution of HER2 receptors between membrane ruffles and flat regions of the cell by internalization and recycling processes. To account for the heterogeneity in cell size and HER2 expression in SKBR3 cells, the dynamic model was expanded to a mixture model. The model describes the experimental observation that drug induced receptor internalization occurs preferentially in cells containing membrane ruffles, while internalization in non-ruffled cells happens at a much smaller rate. Our analysis shows that the common hypothesis of constitutive HER2 recycling back to the plasma membrane is not supported by the data.

Keywords: Modelling, Breast Cancer

SBMC18-55

Lipid Droplet regulation in the liver: The role of Rab18

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Rab18 is a member of the Ras/Rab-family of small GTPases, which has been shown to interact lipid droplets (LDs). Yet, the role of Rab18 on LDs and mechanisms of localization remain largely unknown. In particular, we investigate the role of Rab18 in the liver, since LD formation in hepatocytes is the most conspicuous phenotypic marker of liver steatosis. Localization of small GTPases to membranes occurs through C-terminal reversible palmitoylation which provides dynamic specificity and irreversible prenylation. This prenylation has been linked to the inherent membrane affinity in the Rab/Ras-family members H- and N-Ras. Rab18 is post-translationally geranylgeranylated at C203, and palmitoylated at C199. These hydrophobic modifications are expected to provide the membrane anchors through which Rab18 gains its affinity to LD membranes.
Using ectopic expression of fluorescent Rab18 fusion proteins in hepatocytes, we confirmed that Rab18 localizes to LDs in liver cells. FRAP studies showed that Rab18 localization to LDs is dynamic which indicates a circulation of the Rab18 protein from the ER to the LDs Investigating the role of Rab18's LD localization in liver cells, we created the Rab18 mutants C199S and C203S, which are each mis-localized to the endoplasmic reticulum and cytoplasm respectively. Thus, Rab18 C-terminal hydrophobic modifications are an important determinant in localizing Rab18 to LDs.
We further expressed the constitutively active Rab18 Q67L C199S double mutant in HepG2 cells, which additionally to mis-localization prohibited the formation of large LDs under oleic acid supplementation. These results provide the preliminary mechanistic insight into the context of Rab18's role in controlling on LD composition, size and stability.
Indeed, immunofluorescence staining of Rab18 in human liver biopsies of patients with steatotic pathology (NAFLD, NASH) show complete disruption of Rab18 localization compared to normal liver tissue. Pharmacological control of Rab18 localization and thereby of LD dynamics represents a promising avenue for the eventual clinical management or reversal of liver steatosis.

Keywords: Rab18, Lipid Droplets, Liver

SBMC18-93

Stochastic sequestration dynamics can act as intrinsic noise filter in signaling network motifs

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In this study we investigate the role of cascading for coping with intrinsic noise due to stochasticity in molecular reactions. We use stochastic modeling approaches to quantify fluctuations in the terminal kinase of phosphorylation-dephosphorylation cascade motifs and demonstrate that cascading highly affects these fluctuations.

We show that this purely stochastic effect can be explained by time-varying sequestration of upstream kinase molecules. In particular, we discuss conditions on time scales and parameter regimes which lead to a reduction of output fluctuations. Our results are put into biological context by adapting rate parameters of our modeling approach to biologically feasible ranges for general
binding-unbinding and phosphorylation-dephosphorylation mechanisms. Overall, this study reveals a novel role of stochastic sequestration for dynamic noise filtering in ubiquitous signaling motifs.

Keywords: Phosphorylation-dephosphorylation cycle, sequestration, noise filtering, stochastic modeling

SBMC18-72

Reaction-diffusion based focusing of local cell contraction pulses

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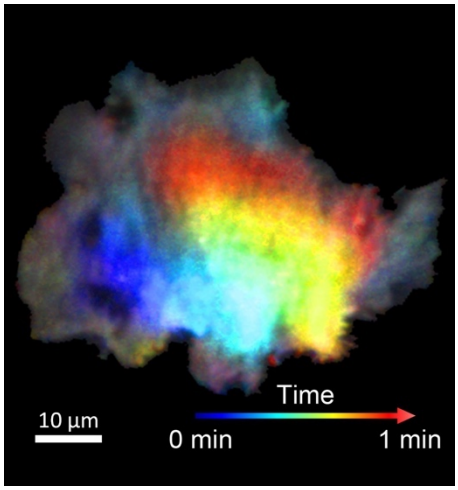
Pulsatile, local contractile forces play important roles during embryonic development. Our recent studies revealed that such contraction pulses emerge from an excitable Rho/myosin-based signal network, that combines positive and negative feedback mechanisms, and that this dynamic behavior is modulated by mechanical properties of the cellular environment. Here, we used photochemistry and optogenetics to apply direct, acute, experimental manipulations of critical components of this signal network to study their function in the generation of spatio-temp cell contraction patterns. From this experimental data we derived a theoretical concept for signal network dynamics. Analysis of this network predicted Rho amplification dependent switching of system dynamics between excitable, oscillatory and stable states that was confirmed experimentally. An extended theoretical concept that includes diffusion of the active and inactive system components can explain the emergence of spatially focused cell contractility patterns by an additional long-range negative feedback due to substrate depletion. Our study can therefore explain how contractility pulses are focused at the subcellular level. We propose that these pulses form the basis of an active, exploratory process that can locally probe the elasticity of the extracellular environment.



Rho activity wave propagation in a U2OS cell. Rho activity was measured via TIRF imaging of effector domain recruitment to the plasma membrane. The temp evolution of a single activity wave is represented by colors (propagation from blue to red).

Keywords: RhoA contraction excitable network optogenetics oscillations simulation myosin

SBMC18-61

Comprehensive transcriptomics mining identifies Gpnmb as a consistently upregulated gene in NASH-based HCC

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Progression of metabolic-based liver disorders to hepatocellular carcinoma (HCC) is clinically well reported worldwide. Since HCC is an untreatable disease, there is need to seek for targets critically contributing to during disease progression to develop a therapeutical intervention. Data mining of non-alcoholic steatohepatitis (NASH) and HCC from patients and mice represents a powerful tool to identify potential candidates in an unbiased way. In that line, we investigated publically available GSE48452 and GSE14520 datasets representing NASH and HCC in patients, and matched these data with the corresponding disease stages in a NASH-based HCC mouse model (STAM) driven by a combination of diabetes and a high fat diet.
Benjamini-Hochberg adjusted P values ($p<0.05$) and log fold changes (± 0.5) were used to extract significantly and consistently altered genes in comparison with the corresponding healthy subjects. Several targets were consistently upregulated i.e. Lgals3, Gpnmb, Sptan1 and Rrm1 in NASH and HCC in patients and mouse. Interestingly, only Atg2a was downregulated in patients and mouse. Among the upregulated genes, glycoprotein non-metastatic melanoma b (Gpnmb) - a transmembrane glycoprotein- was upregulated in several patient cohorts i.e. GSE28619, GSE14323 and GSE6764 representing alcoholic hepatitis, cirrhosis and cirrhosis-based HCC. Immunohistochemistry analyses revealed that GPNMB localizes in parenchymal cells in healthy livers. However, it is detected - in addition - in non-parenchymal cells in case of NASH and HCC. Morphologically, the GPNMB positive cells are obviously macrophages. In conclusion, GPNMB is a consistently upregulated target during liver disease progression. Further investigations to characterize and dissect the role of GPNMB during NASH-based HCC are ongoing.

Keywords: Gpnmb, NASH, HCC

SBMC18-86

Structure-based dynamic modeling reveals drug combinations to overcome oncogenic RAS-to-ERK signaling

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The RAS/RAF/MEK/ERK pathway is pivotal for cell proliferation and survival and is frequently hyperactivated in tumors. Oncogenic mutations in the RAS genes are one of most frequent oncogenic mutations in cancer. Despite long effort at developing RAS inhibitors, there is still no clinically available drug. As a result, the development of inhibitors of the kinases downstream of RAS has become a hot topic in drug development.
Clinically used RAF inhibitors are ineffective in RAS-mutant tumors, enhancing homo- and heterodimerization of RAF kinases, and leading to paradoxical activation of ERK signaling. Numerous mechanisms of RAF inhibitor resistance result in enhanced RAF dimerization and cannot be overcome by existing RAF inhibitors. A way to overcome resistance is the use of inhibitor combinations, but it is unclear how the best combinations can be chosen.
Using a combined experimental and computational approach, we have built a mechanistic ERK pathway model that integrates thermodynamics and kinetics of drug-protein interactions, structural elements, post-translational modifications and cell mutational status to faithfully predict RAF inhibitor responses at the network level. Our model predicts a number of unexpected and hidden properties of network responses to different types of RAF inhibitors and makes new strides in understanding resistance to these drugs. The model suggests that synergy can emerge between Type I and Type II, as well as between Type I 1/2 and Type II RAF inhibitors and predicts new ways of overcoming RAF inhibitor resistance in RAS mutant cells.
To test model predictions, we experimentally measured responses of MEK/ERK signaling and cell proliferation to different RAF inhibitor types and their combinations in melanoma cells bearing oncogenic RAS, BRAFV600E mutations, or both BRAFV600E and RAS mutations. The level of MEK and ERK phosphorylation was measured by means of Western Blot, MESOSCALE and Luminex immunoassay kits. Cell proliferation was measured in MTS and colony forming assays. Our experimental results corroborated model predictions, showing that two RAF inhibitors ineffective on their own can robustly suppress ERK pathway when used in combination.
Our results suggest a new principle of targeting the same kinase with two structurally different inhibitors that bind to

different kinase conformations.

Keywords: RAF, RAS, kinase inhibitors, resistance, mathematical modelling

SBMC18-101

A computational model of EGFR receptor trafficking

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[3]*BMCV, University of Heidelberg and DKFZ Heidelberg (Heidelberg)*

The epidermal growth factor receptor (EGFR), belongs to the ErbB family of transmembrane receptors. It is involved in the regulation of several key cell fate decisions, including proliferation, differentiation, migration and apoptosis. Due to the importance of these processes and the delicate nature of the involved signaling, it is of no surprise that the EGF-receptor and the signaling cascade initiated by it are often found to be modified in tumors. EGFR is hence the target of ongoing and intensive research and has become one of the best investigated receptor tyrosine kinases (RTKs).
While EGF signaling has been extensively researched and several excellent and detailed computational models have been developed to study its dynamics, receptor trafficking and its regulation have come into focus only relatively recently. However, it is well known that EGFR trafficking affects its signaling, as the ligand-receptor complex remains activated and able to initiate new signaling cascades after its internalization. We were interested in whether differences in receptor trafficking can contribute to the carcinogenesis of cervical cancer induced by a chronic infection with human papillomavirus (HPV). Using quantitative
3D-micoscopy, we tracked receptor internalization under different conditions, providing detailed quantitative and dynamic data on EGFR trafficking. Based on these data, we developed a mathematical model of receptor internalization and its further degradation or recycling. Our model confirms the hypothesis that the mode of endocytosis of the ligand-receptor complexes influences receptor fate, and thus affects the intensity and duration of the downstream signaling process. Using data of HeLa cells transfected with an additional HPV oncogene (E5), first results indicate that the oncogene indeed alters EGFRs trafficking process and thereby its downstream signaling.

Keywords: Modeling; EGFR; Trafficking;

SBMC18-32

Systemic transcriptomic characterization of slow and fast liver fibrosis progression in mice

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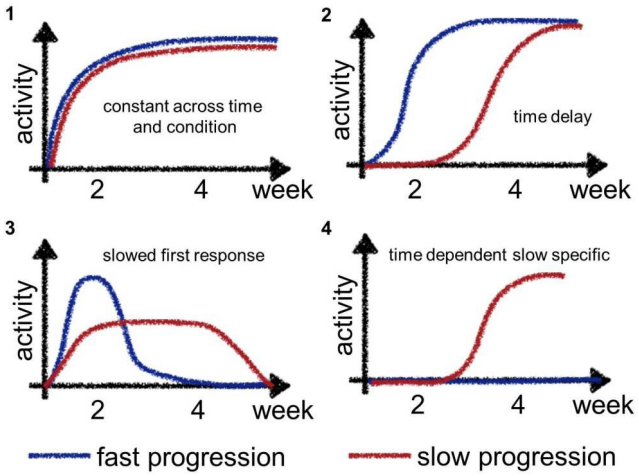
Background and aims: Chronic liver injuries trigger inflammation and wound healing responses resulting in liver fibrogenesis as end stage of chronic liver disease. While morphological alterations are quite similar, dynamics of disease progression are highly variable between patients, and markers to prospectively predict disease progression are still elusive. In this study, we set out to identify relevant and differentially activated pathways and transcription factors with the use of two transcriptomic data sets of murine toxic liver injury models with slow respective fast progressive liver fibrogenesis.
Methods: Liver fibrosis was induced in C57BL/6 mice by repetitive application of the toxic agent carbontetrachloride (CCl4). Slow fibrosis progression was induced using a dosing of 1,0 g/kg body weight, while fast progression was achieved with a dosage of 1,6 g/kg bw (all 2x weekly, i.p.). Mice were analyzed at 0, 2, and 4 weeks. RNA was isolated from the liver and a microarray was performed using the Affymetrix platform. Data were analyzed using the functional genomics tools PROGENy, SPIA and DoRothEA.
Results: Gene expression analysis revealed that both, fast and slow liver fibrosis progression models show an elevated number of regulated genes after 2 and 4 weeks compared to baseline week 0. The number of downregulated genes is comparable between both models, whereas the number of upregulated genes is distinctly higher in the slow progression model. Application of pathway analysis tool SPIA, which combines gene set enrichment analysis and a topology-based approach, revealed 4 different cases of dynamic pathway response between both models (see figure). Surprisingly, the data acknowledge signaling pathways that are time dependent and specific for the slow progression model (case 4) e.g. activation of TGF-beta signaling pathway. These results are supported by PROGENy, a tool which analyses pathway activity from gene expression data. Significant differences between both models are determined in the JAK-STAT pathway and the TGF-beta pathway. Transcription factor analysis was done using DoRothEA showing no direct differences between both models, but revealed notably activated transcription factors with constant activation

over the selected conditions.
Conclusions: In this study, we systemically profiled signaling pathway alterations between fast and slow progression in experimental liver fibrosis models. These data will set the stage to assess the predictive value of the identified alterations in these pathways for human liver fibrosis progression



Keywords: Liver, Fibrosis, TGF-beta, Progression, genomic tools

Metabolism

SBMC18-14

Integrating Modeling of Metabolism and Signaling towards an application in liver cancer

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The etiology of uncontrolled proliferation of mammalian cells is multi-factorial and frequently associated with synergistic adaptations in both signaling and metabolism. As such, dysregulated metabolism and altered signaling are two major hallmarks of cancer. It remains to be clarified how signaling and metabolism give rise to cooperative enhancement and how to best exploit this knowledge for treatment design. Cancer cells typically exhibit the Warburg effect, i.e., a high rate of glycolytic fermentation despite the presence of oxygen. Altered Hepatocyte Growth Factor (HGF) signaling and aberrant Met receptor
activation have been implicated in human malignancies. Furthermore, a role for HGF has been implied in the context of resistance to targeted tumor therapy and metastasis.
When exposed to HGF, the HepG2 liver cancer cell line increases its lactate production flux. To study the driving forces behind this increase, we established a new model of cancer cell glycolysis, which was parameterized on a combination of literature data and metabolic data acquired in HepG2 cells. During model construction, care was taken to preferentially use data specific for those enzyme isoforms typically encountered in the cancerous state. To capture the effect of HGF, several key players in the HGF signaling cascade were monitored in the presence and absence of inhibitors.
The modeling reveals that in HepG2 glycolytic flux control is mostly in the first enzymes and HGF predominantly exerts transcriptional control and not post-translational control over controlling steps in cancer glycolysis via ERK. By integrating both signaling and metabolic data, we assess which interactions play a regulatory role in the HGF induced up-regulation of lactate production. Moreover, the presented model provides a building block for further development for other signaling pathways and tissue types. Future work will be to design an effective combinatorial treatment for down-regulating the glycolytic flux specifically in cancer cells. By using a similar approach for primary human carcinoma cells, we aim to prevent side-effects of future treatments.

Keywords: metabolism, signalling, cancer

SBMC18-33

Metabolic adaptations and therapeutic effects: structural and parametric uncertainties in modelling from longitudinal data

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The study of progressive adaptations during disease or intervention is complicated by the multilevel aspects of the biological system. One of the challenges is to improve understanding of these adaptations by integrating data from the different molecular levels with mathematical models. Two major types of uncertainty need to be addressed in mechanistic mathematical models. Model parameters might not be identifiable from experimental data resulting in uncertainty associated with the quantification of kinetic parameters. In addition, lack of knowledge about components, interactions, and their quantitative features introduce uncertainty about the model structure.
We developed a computational approach called ADAPT that addresses these challenges by introducing time-dependent model parameters. The estimation of dynamic parameters depends on the mathematical model, the experimental data, and additional regularization functions. The latter provides the possibility to integrate additional assumptions about the adapting biological system, without the necessity to explicitly formulate these in the mathematical model. Here we show how regularization within the ADAPT framework can be used to integrate gene expression data from longitudinal studies. This problem concept was formulated as a multi-objective optimization problem in which the estimation of parameter trajectories from time-series metabolomics data is guided or constrained by the gene expression data.
The computational approach was applied to a model of hepatic lipid and plasma lipoprotein metabolism to predict which metabolic adaptations are induced upon pharmacological treatment of mice by Liver X receptor (LXR) agonist T0901317. LXR agonists exert potent antiatherosclerotic actions but simultaneously induce excessive triglyceride (TG)

accumulation in the liver. We revealed that both input and output fluxes to hepatic TG content are considerably induced on LXR activation and that in the early phase of LXR agonism, hepatic steatosis results from only a minor imbalance between the two. It is generally believed that LXR-induced hepatic steatosis results from increased de novo lipogenesis (DNL). In contrast, ADAPT predicted that the hepatic influx of free fatty acids is the major contributor to hepatic TG accumulation in the early phase of LXR activation. This prediction could be validated by a metabolic tracer experiment showing a 5-fold increase in the contribution of plasma palmitate to hepatic monounsaturated fatty acids on acute LXR activation, whereas DNL was not yet significantly increased.
This study illustrates that complex effects of pharmacological intervention can be translated into distinct patterns of metabolic regulation through ADAPT modeling and that implicit integration of gene expression data effectively constrained and improved model predictions.

Keywords: systems pharmacology, uncertainty analysis, longitudinal data, lipoprotein metabolism, liver, LXR, hepatic steatosis

SBMC18-87

Altered glyoxylate metabolism in non-alcoholic fatty liver disease (NAFLD) - a molecular link to kidney injury?

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Non-alcoholic fatty liver disease (NAFLD) is the most common cause of liver diseases in Western countries. It is frequently considered as the hepatic manifestation of the metabolic syndrome, and represents a major risk factor for developing more severe liver diseases, such as cirrhosis and hepatocellular carcinoma, as well as cardiovascular and chronic kidney diseases. The molecular mechanisms linking fatty liver with adverse progression and with damage to other organs are not yet understood. Our study aimed to explore how steatosis-associated changes in hepatic metabolism influence other organs. Based on an integrated epigenome and transcriptome analysis of the fatty liver of the ob/ob mouse NAFLD model, the Alanine glyoxylate aminotransferase (Agxt) was identified as an interesting candidate showing downregulation linked to promoter hypermethylation. Agxt plays a crucial role in glyoxylate detoxification, preventing its conversion to oxalate, which upon excretion can lead to calcium oxalate deposition in the kidney that ultimately cause renal failure. For this reason, we explored whether downregulation of Agxt in the fatty liver results in excessive generation of oxalate, which may contribute to a higher risk of kidney stone formation. Our results showed that primary hepatocytes isolated from the steatotic liver of ob/ob mice displayed an altered glyoxylate metabolism compared to control hepatocytes and released more oxalate when exposed to its precursor
hydroxyproline. Plasma and urinary concentration of oxalate are currently being evaluated, also in other mouse models of NAFLD. In human hepatocytes expression of AGXT was inversely correlated to the degree of triglyceride accumulation and associated with hypermethylation as well. In a cohort of children with NASH preliminary results indicate a correlation between the degree of steatosis and the daily excretion of oxalate. In conclusion, alterations in the expression of genes involved in glyoxylate detoxification in NAFLD may result in a higher production of oxalate and therefore predispose to calcium oxalate stone formation.

SBMC18-59

SABIO-RK: kinetic data for systems biology

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SABIO-RK (*http://sabiork.h-its.org/*) is a manually curated database containing biochemical reactions and their kinetics together with their specifications (experimental conditions, organism, EC number etc.). It is a valuable resource for modellers simulating biochemical networks as well as for experimentalists interested in enzymatic activities and reaction properties. Kinetic data of metabolic, transport and signalling reactions included in SABIO-RK are mainly derived from literature, but also from SBML encoded models or directly from lab experiments. More than 22.500 database entries (38 % of all entries) refer to mammalian, therof 10.800 to human only. Several ontologies are embedded as Gene Ontology (GO) for cell locations and signalling events, Systems Biology Ontology (SBO) for kinetic laws and parameters and the Brenda Tissue Ontology (BTO). The predominant tissue arising in SABIO-RK is the liver with >8.800 entries (15.4 %) thereby giving information not only about wildtype but also mutated proteins. The data in SABIO-RK contain annotations to controlled vocabularies and are highly interlinked with other databases (KEGG, UniProt, BRENDA, Pubmed, ExPASy etc.).
SABIO-RK can be accessed via an easy-to-use web interface or programmatically via RESTful webservices or Python

scripts. Search results can be exported in different formats including SBML, XML and as spreadsheets. Actually SABIO-RK is accessed mainly (about 90 %) via web services, which underlines the importance of its integration into modelling and visualization tools like CellDesigner, VirtualCell, Sycamore, SBMLsqueezer, cy3sabiork, Path2Models, LigDig and FAIRDOMHub.
As part of de.NBI (German Network for Bioinformatics Infrastructure) we offer user support as trainings, knowledge exchange via google groups and the manual extraction of kinetics data from the literature upon user requests.

Keywords: SABIO-RK, enzyme kinetics, modelling, biochemical reactions, database

SBMC18-79

Whole metabolome profiling identifies the combination of an eicosanoid, a bile acid and an androgen as a highly accurate marker of liver fibrosis in patients with non-alcoholic fatty liver disease

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) is among the most frequent causes of liver disease and estimates based on imaging and biopsy studies suggest that about 20-30% of adults in the United States and Europe have excess hepatic fat accumulation, and are at risk of developing progressive fibrosis, end-stage liver disease or hepatocellular carcinoma. Liver biopsy is considered the gold standard in the diagnostic evaluation of patients with suspected NAFLD, and fibrosis at index biopsy identifies those at risk of progression. However, liver biopsy is uncomfortable, more expensive than most non-invasive tools and carries a procedure-related risk which together render it unsuitable for regular screening. This study aims to identify novel non-invasive markers by metabolite profiling that correlate with histological key lesions of progressive NAFLD.
Methods: In this multicenter cohort study, 74 patients with biopsy-proven NAFLD (NAFL, n=42; NASH, n=32) and 62 healthy blood donors were enrolled. Among the NAFLD patients, 13 had advanced fibrosis (>=F2) while the remaining 61 showed absent or mild fibrosis (F0-F1). Obtained EDTA plasma samples were subjected to metabolite analysis by the GC-MS- and LC-MS/MS-based MxP® Broad Profiling approach, and by the targeted platforms MxP® Steroids, MxP® Lipids and MxP® Eicosanoids. The metabolic data were statistically analyzed by applying univariate (analysis of variance) as well as multivariate (random forest, elastic net, and linear discriminant analysis) algorithms, and peak signals were compared to key histology lesions of NAFLD, i.e. fibrosis, activity, and steatosis.
Results: A multi-marker panel, consisting of an eicosanoid, a bile acid and an androgen, successfully differentiated the EDTA plasma samples into two main clusters: fibrosis stage >= 2 and fibrosis stage < 2 (AUC=0.95, Sens=0.92, Spec=0.90, PPV=0.225, NPV=0.997). Thus, our biomarker outperforms the FIB4 index (AUC=0.80), the ELF score (AUC=0.82), and plasma levels of caspase-cleaved keratin 18 fragments (AUC=0.68).
Conclusions: The identified metabolite panel was significantly associated with liver fibrosis stage in NAFLD patients and reveals an excellent test performance to detect early prognostic histological liver lesions. In sum, this biomarker holds high promise to accurately detect fibrosis in NAFLD patients non-invasively in plasma, and thus may serve as a novel non-invasive screening tool in NAFLD patients.

Keywords: NAFLD, FIBROSIS, EARLY BIOMARKER, METABOLISM

SBMC18-28

Functional intravital two-photon based imaging of bile salt transport in cholestasis: mechanisms and consequences of bile infarct formation

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Background and aims: The mechanisms of parenchymal damage in cholestatic liver disease remain unclear. Therefore, we studied the spatio-temp processes of bile salts (BS) leakage from the biliary tract and aimed to understand its pathophysiological role.
Methods: Intravital two-photon-based imaging of common bile duct-ligated (BDL) mice was performed with fluorescent BS and non-BS organic anion analogues. Key findings were followed-up by MALDI imaging, clinical chemistry, immunostaining and expression analyses.
Results: In the acute phase after BDL, BS concentrations in bile are increased and single cell bile micro-infarcts occur caused by apical hepatocyte membrane rupture after loss of mitochondrial membrane potential. This leads to cell death by entry of bile, followed by a 'domino effect' of further death of neighboring hepatocytes. Bile infarcts provide a trans-epithelial shunt between bile canaliculi and blood by which bile constituents leak into blood. In the chronic phase, uptake of BS tracers at the sinusoidal hepatocyte membrane was reduced, which contributes to elevated concentrations of BS in blood and decreased concentrations in the biliary tract.
Conclusions: In the acute phase after BDL bile micro-infarcts occur in a limited number of dispersed hepatocytes followed by larger infarcts involving neighboring hepatocytes. These infarcts allow leakage of BS and other solutes from the biliary tract into blood, thereby reducing their concentrations in the canalicular network. This protects the liver from BS overloading. In the chronic phase after BDL reduced sinusoidal BS uptake appears to be a dominant protective factor. The kidney contributes to the elimination of BS until cholemic nephropathy sets in.

Keywords: Intravital imaging; Bile duct ligation; Bile salt transport; Membrane rupture; Bile canaliculi.

SBMC18-71

Integrating modeling of metabolism and signaling towards an application in liver cancer

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One of the major hallmarks of cancer is the uncontrolled proliferation of cells. Rapidly proliferating tumor cells steadily require energy and building blocks for growth. Additionally, in the context of liver cancer, activating mutations or over-expression of receptor tyrosine kinases, such as hepatocyte growth factor receptor (MET), are frequently observed. However, the interaction of the underlying regulatory networks is still poorly understood.
The aim of the project is to determine differences in the signaling-metabolism network in cancer and healthy liver cells. Moreover, we aim to use integrative mathematical modeling to predict potential therapeutic targets. Therefore, we tested the impact of the ligand of the MET receptor, Hepatocytes Growth Factor (HGF), on the hepatoma cell line (HepG2) and primary human hepatocytes (PHH) and observed the response at protein and mRNA levels. Additionally, to disentangle the influence of the PI3K pathway and the MAPK pathway on the induction of gene expression, inhibitors blocking these pathways were applied.
It was observed that in both cell types HGF treatment activated the MAPK and the PI3K signaling pathways, however mRNA expression of glucose transporter type 1 (Slc2a1) was regulated via MAPK pathway in HepG2 cells and not in PHH. Inhibition of the PI3K and the MAPK pathways in PHH did not affect the HGF-induced expression of glucose transporter type 3 (Slc2a3) but the inhibition of the MAPK pathway in HepG2 cells resulted in a loss of expression of Slc2a3. Only in HepG2 cells the AKT inhibitor alone already induced the mRNA expression of Slc2a1, Slc2a2 and HK2 genes. Modelling the integrated system, we found that in our mathematical model of HepG2 signaling and metabolism, the glycolytic flux was mostly controlled by those enzymes which were transcriptionally upregulated by the MAPK pathway. In the model, we captured the negative control exerted by AKT in HepG2 via an inhibitory effect upstream of ERK. This in combination with a high basal activation in HepG2 allowed us to describe the behavior in both cell types. With these steps, a first framework was established to generate data for the development of an integrative modeling approach for signaling and metabolism which could provide us with novel interventional strategies to target liver cancer.

Keywords: Metabolism, Signaling, Modeling, Liver Cancer

SBMC18-96

Along the porto-central axis - Zone specific isolation of primary hepatocyte and its integration into a metabolic model

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The maintenance of liver homeostasis relies on metabolic zonation of the liver lobulus, where the pericentral and periportal zones differ in their enzyme composition, content and activity. However, the exact mechanisms are not fully understood. Thus, to carry out a proteomic and metabolic fingerprint along the porto-central axis of the sinusoid is of high relevance for establishing a suitable systems biology approach aiding a better mechanistic understanding of hepatocytes heterogeneity with their different cellular functions.
In the past the gold standard method for isolation of periportal and pericentral hepatocytes was a Digitonin perfusion of the liver by which the hepatocytes of either periportal or pericentral area are destroyed. Subsequently, a collagenase perfusion is performed which results in the extraction of the remaining cells. Unfortunately, this method has some disadvantages, namely the possible bleedthrough of undesired cells with the consequential loss of purity and the poor predictable amount of disrupted hepatocytes along the porto-central axis. Another problem is that in most cases only one of the two populations (periportal vs. pericentral) can be obtained via the Digitonin perfusion.
Here, we present a new method for isolation of periportal and pericentral located hepatocytes, which is based on zone specific antigen detection followed by sorting via flow cytometry. The ad-vantages of this method are a high purity of both hepatocyte populations due to zone specific anti-gen detection. In addition both populations and zones of different size can be isolated from one donor depending on the chosen antigens. After flow cytometry a proteomic analyses was per-formed and the data was integrated into a metabolic model.

Keywords: Flow Cytometry, Hepatocytes, priportal, pericentral, metabolic model

SBMC18-17

Thrombin signal inhibition via PAR4-Deletion improves metabolic status and reduces NAFLD in mice fed high-fat diet

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Overweight, diabetes, and metabolic syndrome are the main risk factors for developing non alcoholic fatty liver disease (NAFLD). About one-third to one-half of type II diabetics suffer from NAFLD. Recent investigations revealed that the development of NAFLD is not only an accompanying phenomenon of type II diabetes. The increased accumulation of fats in the hepatocytes appears to be rather directly related to the development of insulin resistance and pathological glucose tolerance. NAFLD plays therefore a pathogenically relevant role in the development of type II diabetes. Underlying this observation the molecular mechanisms are poorly understood. Different studies postulate that certain messengers (adipokines) are of particular importance in this context. The G protein-coupled thrombin receptor PAR4 is expressed on various cell types, e.g. thrombocytes, endothelial cells, smooth muscle cells, but also on adipocytes. Increased thrombin activity in adipose tissue has been associated with inflammatory macrophage recruitment and development of insulin resistance in mice. The contribution of PAR4 to metabolic and inflammatory changes in the course of diabetes and NAFLD development has not been reported to date. We reveal that HFD-fed wildtype mice showed 10-fold upregulated PAR4 expression, particularly in WAT, compared to chow-fed controls. In PAR4-deficient mice, HFD resulted in less weight gain, lower body fat mass and smaller WAT fat pads than in wildtype mice. Average adipocyte areas in both WAT and BAT was also reduced in PAR4-/- vs. wildtype mice fed HFD, as were CLS by 85%. HFD-induced impairment of glucose tolerance and insulin sensitivity was significantly worse in wildtype vs. PAR4-/- mice. These alterations coincided with a significant reduction of lipid accumulation in the liver in PAR4-/- mice. Both in liver and WAT expression of the proinflammatory cytokine CCL2 is downregulated in PAR4-/- mice compared to wildtype controls. This preliminary study highlights PAR4 as a candidate regulator of adipose tissue inflammation and metabolic dysfunction in the development of diabetes and NAFLD.

Keywords: thrombin, PAR4, NAFLD, adipose tissue, inflammation

SBMC18-76

A Biochemistry-Based Kinetic Model of Liver Metabolism for Applications in Medicine and Pharmacology

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The epidemic increase of non-alcoholic fatty liver diseases (NAFLD) requires a deeper understanding of the regulatory circuits controlling the response of liver metabolism to nutritional challenges, medical drugs and genetic enzyme variants. As in vivo studies of human liver metabolism are encumbered with serious ethical and technical issues, we developed a comprehensive biochemistry-based kinetic model of the central liver metabolism including the regulation of enzyme activities by their reactants, allosteric effectors and hormone-dependent phosphorylation. The utility of the model for basic research and applications in medicine and pharmacology is illustrated by simulating diurnal variations of the metabolic state of the liver at various perturbations caused by nutritional challenges (alcohol), drugs (valproate) and inherited enzyme disorders (galactosemia). Using proteomics data to scale maximal enzyme activities, the model was used to highlight differences in the metabolic functions of normal hepatocytes and malignant liver cells (adenoma and hepatocellular carcinoma).

Keywords: metabolism; mathematical model; kinetic modeling; enzyme kinetics; liver; alcohol metabolism; galactosemia; gene expression; valproate; tumor metabolism

SBMC18-45

The crosstalk of Hedgehog and mTor signaling in Hepatocytes

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The morphogenic Hedgehog (Hh) pathway is crucial for embryonic development and tissue differentiation. However, it is also an elementary regulator of homeostasis in adult tissues, although its activity decreases significantly during adolescence. Previous studies show a connection between Hh pathway and the signaling of the mechanistic target of rapamycin (mTor), especially in cancer. mTor is a serine/threonine protein kinase which forms two different complexes mTORC1 and mTORC2. Through the phosphorylation of various down-stream molecules, mTor stimulates essential metabolic pathways like mitochondrial
metabolism, autophagy, cell growth and survival, aging, cancer progression and nutrient metabolism. We observed the repression of the mTor pathway in hepatocytes with deleted Hh signaling, suggesting a crosstalk between Hh pathways and mTor signaling in the liver.
To investigate the crosstalk of Hh and mTor signaling in hepatocytes, various hepatocyte-specific knockout mouse models have been bred for activation and inactivation of Hh signaling. Inactivation was carried out by deletion of Smoothened (Smo), which is an important receptor protein in the Hh signaling cascade. Hh activation was achieved by inhibition of the receptor protein Patched 1 (Ptch1). For further analysis of the direct influence of the inhibition and activation of the Hh and mTor signaling on hepatocytes, primary hepatocytes from C57Bl/6N mice were isolated and treated with insulin to activate the mTor pathway and cyclopamine to inhibit the Hh pathway. The primary hepatocytes from all sources were further analyzed using immunohistochemistry, Western Blot, qPCR and Seahorse technology.
The results of our studies show a Hh dependent activation of mTor in hepatocytes. Hepatocytes from Smo Knockout mice show a decrease in the phosphorylation of mTor signaling molecules like Raptor, whereas hepatocytes from mice with activated Hh pathway show an increase in the phosphorylation of mTor. In addition mRNA levels of mTor signaling molecules like mlst8, mTor and Raptor are increased in hepatocytes from Smo Knockout mice. Immunohistochemical stainings show, that the activation of Hh not only changes the phosphorylation of mTor, it also alters the location of the phosphorylated proteins in the liver from a
pan-lobular to a solely pericentral position. Furthermore evidence has been found that the alteration in the crosstalk of the Hh and mTor pathway influences glucose metabolism, ATP production and mitochondrial aspiration of cultured hepatocytes.

Keywords: Hedgehog, mTor, hepatocytes

Computational and Experimental Techniques

SBMC18-34

Considering Multi-Specific Enzymes in Metabolic Simulations Using Agent-Based Modeling

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Introduction: Computational modeling of metabolic networks has commonly relied on ordinary differential equation (ODE) systems based on simple mass action, Michaelis-Menten [1], or other specific kinetics. Such models have been successfully applied, e.g. to simulate dynamic system responses, elucidating recurring patterns, oscillations, and steady-states.
Problem description: However, we encountered multiple ODE models which lack consideration of multi-specific enzymes capable of catalyzing various reactions with different substrates, resulting in overestimating enzyme activity. Model fitting may eventually lead to sufficient model behavior, but used kinetic constants are often unrealistic, limiting a model's validity and quantitative inference potential.
Method: We have developed an agent-based (AB) modeling framework that considers multi-specific enzymes. In agent-based modeling systems dynamics are inferred from an emergent behavior of autonomous agents. In our framework agents are single enzyme molecules which exhibit stochastic behavior.
Results: For evaluation we use an ODE model of arachidonic acid metabolism in human neutrophils [2]. While qualitatively similar, the ODE model exhibits much faster dynamics than our AB model (Fig. 1).

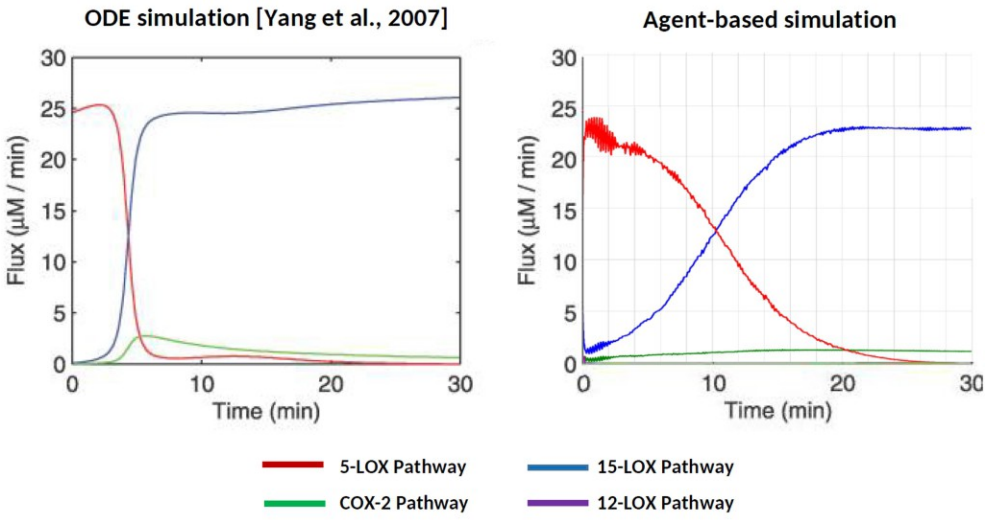

Figure 1: The ODE simulation shows steep flux dynamics, rapidly reaching steady-state fluxes within roughly three minutes. AB results show overall slower dynamics but similar steady-state fluxes.

In contrast to ODE systems which are efficient in large and complex simulations, the Gillespie algorithm [3] excels in low substrate concentrations scenarios down to single molecule tracking, e.g. in single cell models. Comparisons of our AB model to the Gillespie algorithm show only marginal deviations (Fig. 2).

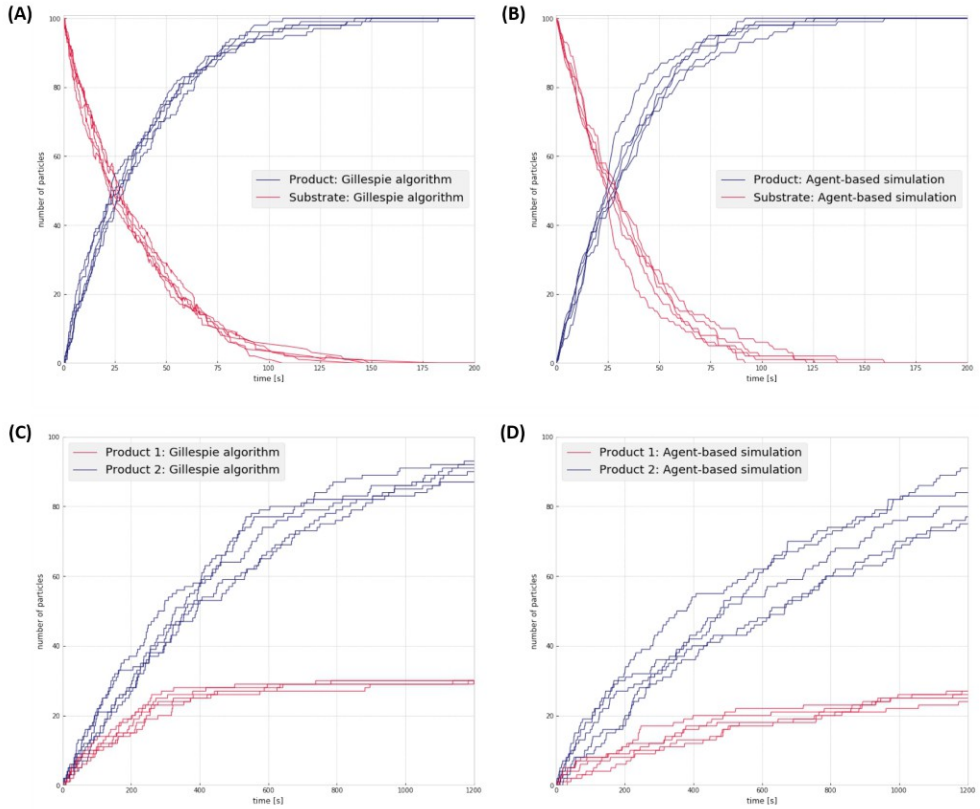

Figure 2: A, C - Gillespie; B, D - AB. A, B show five simulations of an enzyme catalyzing a single reaction: B exhibits slightly higher stochasticity. C, D show an enzyme catalyzing two reactions: D exhibits slightly higher stochasticity and slower dynamics

Conclusion: In our evaluation the AB framework performed with high accuracy in both, small and large scale model simulation scenarios considering multi-specificity, albeit being computationally intense in very large scale and complex scenarios.

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Keywords: Modeling, metabolism, metabolic model, ODE-models, agent-based models, multi-specific enzymes, lipid metabolism, arachidonic acid metabolism

SBMC18-10

Optimal path between parameter estimates in nonlinear ODE systems

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Frequently, ordinary differential equation (ODE) models are used to mathematically represent the dynamic behavior of cellular components, e.g. for signal transduction or gene regulatory networks. For parameter estimation in ODE models, the discrepancy between data and model output needs to be minimized by finding a parameter vector in the high-dimensional search space which is optimal in terms of a minimal value of an objective function. The usage of local deterministic optimizers from multiple initial guesses within the parameter search space typically reveals the existence of several local optima.
For statistically valid conclusions, it is of general interest, whether the appearance of local optima is only a result of a not completely converged local optimizer and may be solved by fine-tuning of the numeric algorithms or if this is a consequence of the local non-convexity of the objective function and hence an intrinsic property of the model and data.

In order to clarify this question in application settings, we present a method for finding an optimal path between local optima on the objective function in the parameter space.
By analyzing the path's profile, i.e., the value of the objective function along the path and its dependency on the parameters, it can be investigated if two optimization results are de facto separated or connected.

Keywords: nonlinear ODE models, Parameter Estimation, Mathematical Modelling, Numerical optimization, Local optima, optimal path

SBMC18-62

Modeling liver volume regeneration with linear elasticity

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Question: The human liver is prone to numerous diseases, with the most common being hepatitis, steatosis, cancer, cirrhosis, alcohol and drug-induced damage. Some of them can be treated by removing the volume that is affected, and allowing the liver to regenerate to nearly full size. In certain conditions, liver cancer is treated through tumour tissue removal, and furthermore, living donor transplants by resection can provide healthy organ tissue for the donor and receiving patient, thanks to the liver's regenerative properties.
The work at hand aims at numerically simulating the volumetric regenerative properties of the liver. The goal is to better understand this regenerative behaviour, and to help plan partial hepatectomy procedures in humans.
Methods: Given experimental data of geometry and hepatocyte proliferation indices following a mouse liver partial hepatectomy, and with the help of image processing techniques, a model of liver regeneration has been implemented as a steady state solution of a linear elastic problem.
First, images of the Bromodeoxyuridine-stained sliced post-resection liver were analyzed to quantify the proliferation of cells. This proliferation has then been analyzed and interpreted as forces, to serve as input for a linear elastic problem solver. The liver's regeneration is finally modeled by being broken down into several intermediate applications of the aforementioned solver.
Results: The regrowth of a partially resected mouse liver over a period of two weeks is simulated. Experimental data of mouse livers at various time points post-resection is used as input to properly specify the rate of growth along this period. Preliminary results show the simulated organ reaching close to the pre-resection volume.
Conclusions: The interpretation of hepatocyte proliferation data as input for a linear elastic problem results in an expected relative growth of the liver, namely restoring it to close to the pre-resection volume.
As future work, if special care is given to a correct corresponding elastic interpretation of the vascular system, its influence on the regrowth process could be simulated by interpreting it as a system of springs. Finally, a rigorous experimental comparison and validation of the simulation could be achieved if measurements of hepatocyte proliferation rates were provided in real-time and
in-vivo. In such a case, this model could then be further developed to help plan partial hepatectomy interventions in humans, in order to increase the recovery prospects.

SBMC18-68

Computational modelling of postprandial glucose and insulin dynamics: the role of amino acids

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Introduction: Amino acids are increasingly recognized as an important factor influencing glucose metabolism. Integrating amino acids in whole-body metabolic models of the postprandial glucose regulatory system is essential to understand the interaction between amino acids and glucose homeostasis. The calibration of these model parameters, however, requires a lot of quantitative data. We propose a combined approach of an extensive literature search and computational modelling to study the effects of amino acids on glucose homeostasis.
Methods: The electronic literature search was conducted in PubMed. A search strategy, consisting of Mesh terms, Boolean operators and Title searches, was employed to identify all relevant articles related to the research question: "What are the quantitative effects of amino acid intake on short term glucose and insulin dynamics in humans?". The search was limited to studies in humans and English language articles. In parallel to the literature search, we extended a computational model of the postprandial glucose regulatory system, termed the Eindhoven Diabetes Education Simulator (E-DES), starting with the BCAA leucine. The E-DES model was created by adjusting and combining different models from the literature [1]. The model consists of several compartments: the gut, the plasma, the interstitial fluid and the subcutaneous tissue. For these compartments the dynamic in- and outflow of

glucose, insulin or both is calculated using (coupled) differential equations.
Results: The initial literature search identified 8987 records. After screening of titles and/or abstract, 8517 records were excluded. The remaining 470 records were clustered based on amino acid/protein type. 25 records contained information on leucine, which we chose for the initial modelling due to it being one of the BCAAs. The E-DES model was implemented in MATLAB 2017b. The differential equations were adjusted to account for leucine, which was given as an input to the model. As a first attempt to optimize the model, we estimated the glucose and insulin parameters to best describe the experimental data. We employed a dataset, identified through the literature search, consisting of glucose and insulin time series data after ingestion of leucine, leucine & glucose, glucose, or water [2]. As a next step we aim to predict the glucose and insulin response for independent datasets containing glucose, insulin and leucine time series data to verify the optimized parameters. Ultimately, we want to integrate all relevant amino acids into the model.

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SBMC18-97

TiSim - A Workbench and Framework for Biophysical Agent-Based Multi-Scale Multi-Cellular Models

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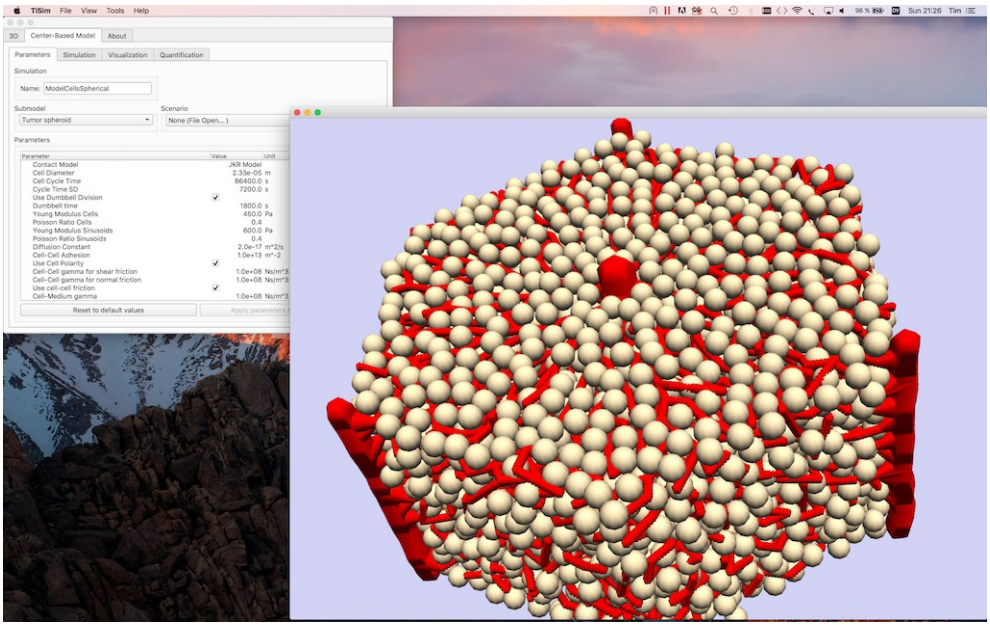
It remains a difficult and resources-consuming challenge to analyse tissue organization processes spanning many scales including the organ scale, functional tissue subunits, cells, down to biochemical reaction networks, experiments by experiments alone. Here, multi-cellular mathematical models are increasingly developing as a fundamental pillar for understanding the mechanisms underlying destructive, degenerative, regenerative or growth processes in living tissue displaying tissue
micro-architecture. Simulations of computational models combining biological and physical aspects can assist by guiding experiments towards the study, in how far a set of hypotheses might be responsible for certain experimental observation, or if a revision is necessary.
The development and application of such models still requires strong computational skills, often over different disciplines.
TiSim was developed collaboratively as a tool to build computational physics-based lattice-free agent-based multi-cellular models involving components on multiple scales. In order to facilitate access to existing models for users without a computational background, TiSim was developed with a graphical user interface (GUI), aiding with setup, parametrisation and simulation control of diverse multi-cellular physics-based models, and providing feedback by featuring near real-time visualisation of the simulated model.
At the same time, TiSim provides the tools to create such models in the form of a C++ framework. The development costs of new models are leveraged by providing the common modules of the models which can be reused or reimplemented and extended.
The data structures readily integrate the models into the GUI, reducing overhead for GUI development.
The models included in this first release of the software encompass not only the regular benchmark cases for centre-based cell models, such as monolayer and tumour spheroid growth, with or without embedding cell medium, but also a multi-scale model of TRAIL-induced apoptosis in a monolayer with cell-individual resilience, and a liver lobule regenerating after CCl4 induced intoxication.

To demonstrate the extensibility of the software we present examples of ongoing work on models exceeding the centre-based models in accuracy and complexity.



A model of liver lobule intoxication and regeneration with parameters in the TiSim GUI.

Keywords: physics-based multi-scale multi-cellular agent-based models, simulations, software, GUI

SBMC18-36

An experimental approach to identify genes that determine hepatocyte polarity

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Hepatocytes are the predominant cell type composing the liver parenchyma. As epithelial cells, hepatocytes polarize forming distinct apical, basal and lateral membrane domains. However, the way how hepatocytes organize these domains spatially is rather unique and has a major impact on the specific architecture of the liver. In contrast to simple epithelia (e.g. bile duct cells in the liver) where the apical surface of all cells is oriented in the direction of the lumen of the organ, the apical membranes of neighboring hepatocytes are juxtaposed to form the bile canaliculi (BC) - a highly ramified capillary network transporting bile towards the bile ducts. The molecular mechanisms which dictate the establishment of hepatocyte polarity and BC are still unknown. To address this, we have developed an experimental strategy to identify genes responsible for the establishment of hepatocyte polarity. We have optimized a culture of primary mouse hepatocyte fetal progenitors, which allows the cells to differentiate and recapitulate the formation of the BC in vitro. At the same time, the culture system supports the establishment of the columnar polarity, enabling us to study genes which might discriminate between hepatocyte and columnar polarity. To test the relevance of candidate genes, we silence them by RNAi during the polarity establishment in the culture system and evaluate the impact on BC formation and morphology. In parallel, we performed a gene expression profiling on
un-polarized progenitors, polarized hepatocytes and columnar bile duct cells in order to find genes, which are specifically enriched in hepatocytes. Finally, we test the candidate genes in vivo by acute gene depletion in adult and embryonic mouse liver, using siRNAs formulated into lipid nanoparticles. This approach led us to the identification of new genes playing a role in the hepatocyte polarity, particularly in the regulation of the bile canaliculi morphology.

Keywords: hepatocyte, polarity, bile canaliculi, in vitro culture

SBMC18-80

Supervoxel-based image analysis for microscopic images

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During the last decade, advances in imaging technology led to an enormous increase in the amount of produced image data. A multitude of microscopy techniques generates a variety of image types such as two-dimensional whole slide scans,
three-dimensional image stacks or time resolved image sequences. In order to obtain segmentations image processing pipelines often are specifically tailored towards a certain image setup, for example in terms of dimensionality and staining. Hence, such pipelines are typically limited in their applicability to differing image setups. Engineering of accordingly adapted pipelines can be a time-intensive process. Additionally, image processing pipelines often require the user to be trained and to have an understanding of the inner workings of the algorithms, which greatly hampers their usability in the wet lab. Therefore, it is indispensable to develop image processing methods, capable of processing a variety of image types and segmenting diverse tissue structures requiring only few user interventions in a unified, easy to use and intuitive image analysis tool that can be used by virtually everyone.
We present a novel image segmentation methodology implemented in our image processing and analysis tool TiQuant that allows untrained users without image processing expertise to segment two- and three-dimensional images in an interactive, nearly parameter-free and standardized way. The tool is used to produce an initial oversegmentation of an image into so called supervoxels, which are analyzed for features such as local, and neighborhood color histograms, texture and gradients. Users are enabled to provide labeled training data for an image dataset through a convenient drawing interface. The features of the labeled data are used to train SVM or random forest classifiers, which are then used to predict class-membership probabilities of the dataset's supervoxels. Segmentations are produced by thresholding of those probabilities and can be post-processed with
size-based object removal, smoothing and watershed filters to remove small artificial objects, clean up boundaries and separate clustered objects.
TiQuant provides a graphical user interface to guide this workflow as well as a batch mode to process a large amount of images using previously trained and saved classifiers. The tool will be freely available.
In the contribution to the SBMC 2018 we will introduce our supervoxel-based image processing module with an emphasis on the underlying methods as well as strengths, weaknesses and potentials of the approach.

Keywords: image analysis; image segmentation, imaging, software tools, image processing

SBMC18-49

Data Needs Structure: Data and Model Management for Systems Biology and Systems Medicine

Martin Golebiewski[1], Olga Krebs[1], Hadas Leonov[1], Stuart Owen[2], Maja Rey[1], Natalie Stanford[2], Andreas Weidemann[1], Ulrike Wittig[1], Katy Wolstencroft[3], Jacky L. Snoep[4], Carole Goble[2], Wolfgang Müller[1]*
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We develop and offer integrated data management support for research in the fields of systems biology and systems medicine within and across research consortia. This support is applied and offered to geographically dispersed, interdisciplinary and large-scale research initiatives in which we are part of, like the German research network 'Systems Medicine of the Liver' (LiSyM*: http://www.lisym.org*), as well as European research networks like ERASysAPP, the former SysMO network (Systems Biology of Microorganisms) or NMTrypI (New Medicines for Trypanosomatidic Infections). Parts of these solutions are also applied to projects with a local focus as the Synthetic Biology Centres at Manchester (SynBioChem) and Edinburgh (SynthSys).
Our data management concept aims at bundling, storing and integrating research assets like data, models and description of processes and biological samples in a Findable, Accessible, Interoperable and Reusable (FAIR) manner (*http://fair-dom.org*) and consists of 4 major pillars:
1. Infrastructure backbone: The SEEK software as registry and a commons for data, models, samples, processes and resulting publications or presentations, at the same time yellow pages for projects, people and events. SEEK is either implemented as data management platform that is maintained by the research project itself (e.g. LiSyM SEEK: *http://seek.lisym.org*) or as hub service maintained by us and spanning different consortia (FAIRDOMhub: *https://www.fairdomhub.org*).
2. Standardized data description: Data spreadsheet templates and tailored use of controlled vocabularies and ontologies to

describe data and metadata
3. Modelling support: Seamless handling and simulation of models by integrated modelling platforms (JWS-Online, SYCAMORE, Cytoscape)
4. Social support: Facilitators (PALs) in the projects for gathering requirements and dissemination
Unlike the majority of data management systems, we specifically support the interaction between modelling and experimentation. Datasets can be associated with models and/or workflows or biological samples, and model simulations can be compared with experimental data.

Keywords: Data management, Databases, SEEK, LiSyM, FAIRDOM, Data integration

SBMC18-54

Method comparison: Impact of Non-dimensionalization on fitting ODEs

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The dynamics of complex biochemical reactions as they occur in living cells can be modeled by ordinary differential equations (ODE). One major task is model calibration, i.e. to estimate parameters like initial concentrations and rate constants based on time-resolved experimental data. Optimization-based estimation like maximum likelihood is often challenging due to the existence of local minima, the highly nonlinear model responses and the limited precision of numerical ODE solutions.
It has been claimed that parameter transformations are beneficial for fitting of ODE models. Possible parametrizations exploit the model's scaling invariance originating from the free choice of units and the fact, that measurements in molecular cell biology are often taken on a relative scale. However, up to now the impact of reparametrization has not been evaluated in detail. To fill this gap, we hereby present a comprehensive method comparison study.
For seven established models of cellular signaling pathways and infectious diseases with available experimental data, we analyze the effect of reparametrization on the performance of optimization. The different influences including the geometry of the likelihood landscape, the choice of initial guesses and the parameter search space are quantified using multivariate statistical analyses.
In these analyses we show that alterations in optimization performance are highly model dependent. Overall, the study suggests an improved optimization. It could be shown that results are transferable between different commonly used optimizers. More studies like this one can help to determine an optimal guidance for choices that are frequently to be made during the modeling process of dynamic ODE systems.

SBMC18-19

A new approach for the reliable assessment of small differences in hepatic steatosis

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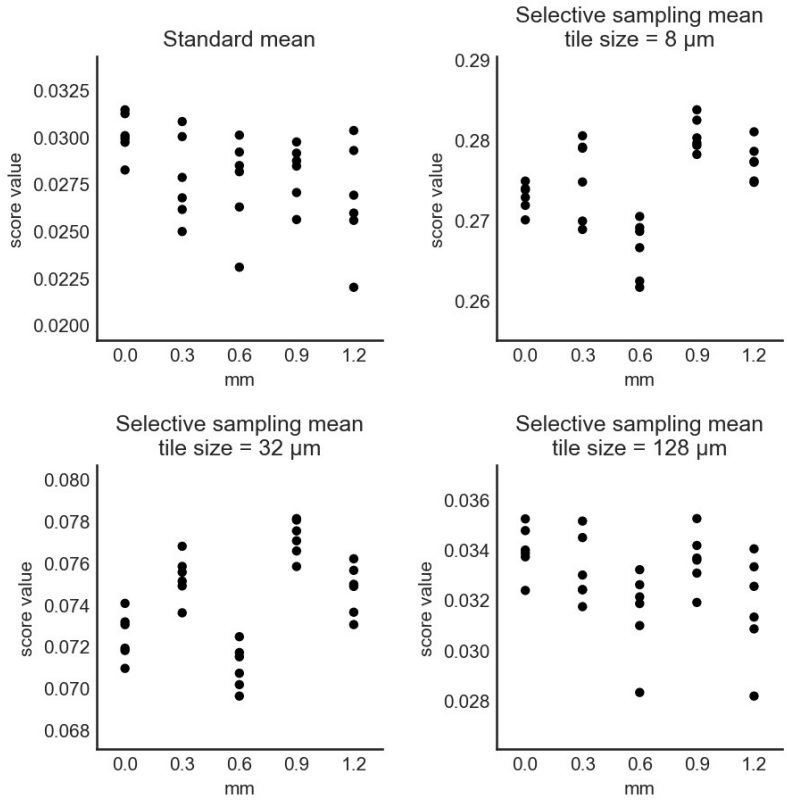
Steatosis is a frequent pathological parameter in models of disturbed hepatic metabolism and drug toxicity. Computerized image analysis enables the automated quantification of steatosis in histological tissue sections. However, the existing methods are generally not accurate enough for a reliable assessment of small differences in steatosis. We evaluated a new image analysis approach that intends to solve this problem through selective sampling of steatotic tissue areas.
Our approach processes hematoxylin-and-eosin (H&E) stained images of liver sections in three steps. First, sections are divided into small tiles and the area fractions of fat droplets in the tiles are determined via automated image analysis. Second, tiles are selectively sampled based on their steatosis area fraction. Third, a steatosis score is computed as the mean steatosis area fraction in the sample.
We evaluated our approach on 30 H&E-stained serial sections of a steatotic rat liver. The sections were divided into five groups, spaced 300 µm apart, of 6 adjacent sections each. It was expected that small differences in the level of steatosis exist between these groups because of intra-liver heterogeneity.
We evaluated multiple variants of our steatosis score that were computed with tile sizes of 8 µm, 32 µm, or 128 µm, respectively. For comparison, we also evaluated the standard score obtained without selective sampling, which corresponds to the result produced by most automated image analysis methods. The reliability of these scores in

distinguishing the different groups was quantified in terms of the intra-class correlation coefficient (ICC).



The standard score proved to be incapable to distinguish the different groups (ICC = 0.14). The reliability of the new scores, on the other hand, depended on the tile size. It ranged from poor (ICC = 0.28, tile size = 128 µm) over good (ICC = 0.72, tile size = 8 µm) to excellent (ICC = 0.83, tile size = 32 µm).
Selective sampling of steatotic tissue areas appears to be a simple way to greatly improve the automated histological assessment of small differences in steatosis, provided that it is applied with the right tile size. This might enable the creation of more precise models of disturbed hepatic metabolism or drug toxicity that consider steatosis as a parameter.

Keywords: steatosis, image analysis, score, reliability, hepatic metabolism, drug toxicity

SBMC18-77

Detecting Critical Transition to Sepsis in Intensive Care Units

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Sepsis manifests itself as a life-threatening response of the body to infections. It is a major challenge to the health care sector worldwide. In Germany, resulting in more than 75000 deaths, Sepsis ranks as the third most frequent cause of death and is a huge health economic burden [1]. Therefore, there is a pressing need for an early diagnosis and treatment of this syndrome. With the availability of large longitudinal observational datasets, it is now possible to develop automatic screening tools which could reduce recognition delays and improve screen accuracy as compared to manual screening [2]. In the present study, we propose a computational framework to detect regions of instabilities in an unsupervised manner in Septic versus non-Septic patient cohorts as per Sepsis 3 definition [3]. We demonstrate that certain clinical variables are significantly different around such regions, which could possibly hint as putative disease driving mechanisms. We demonstrate our method on the multivariate Intensive Care Units (ICU) data obtained from MIMIC-III database [4]. Essentially, we model the putative disease driving mechanism by means of a linear state space model in a rolling time window manner. Subsequently, we compute the regions of instabilities as forecast breakdowns. We find a meaningful intensification of the respective signals before the onset of Sepsis thereby demonstrating the predictive potential of specific patterns indicating instabilities of control in complex systems for Sepsis risk assessment.

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Keywords: Sepsis, Critical Transitions, Disease Progression

SBMC18-91

Multi-omics integration using systematic mapping strategy in NAFLD

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Background and Aims: Nonalcoholic fatty liver disease (NAFLD) is one of the most common diseases with an estimated global prevalence of 25 %. A multitude of studies considering data from different multi omics levels have been performed to reveal its pathogenesis. To assess the results of several studies across multiple omics-levels simultaneously, data integration is inevitable. To map between omics levels we developed biomapr, a flexible R package. To demonstrate the general feasibility of integrating results of several studies in the context of NAFLD, we overlapped NAFLD-associated genes and methylation loci from several studies.
Methods: We collected most commonly used public reference databases from Ensembl, NCBI, Uniprot, HCGN and MGI, including identifiers from genes, transcripts and proteins as well as gene names. So far we assembled 1,1 million molecular identifiers and 2,7 million mappings in a neo4j graph database. On top of this graph data we implemented biomapR to extract mappings in a fast and flexible way. In a pilot study we integrated NAFD-associated genes and methylation loci from four studies (FDR <= 0.1). We overlaid the genome-wide messenger RNA expression, epigenetics data and Methylome-Transcriptome relationships.
Results: We overlapped sets of genes with significantly altered expression and we identified a common set of genes. The additional integration of epigenetic data resulted in a set of five common genes regulated in NAFLD (CACHD1, PBX1, APOA5 ,OAT, MAT1A).
Conclusion: To support and accelerate data integration, we developed the R-package biomapR, an id mapping tool incorporating a multitude of public reference databases (*https://github.com/icb-knowing/biomapR*). In a pilot study, we demonstrated that the integration of NAFLD-related studies considering multi-omics data resulted in a common overlap. This finding should further motivate data integration in other diseases.

SBMC18-52

Modular Response Analysis for non-modular networks

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The molecular networks considered in systems biology are characterized by direct molecular interactions such as activation of an enyzme via posttranslational modifications by another protein. Since a complete map of existing molecular interactions is missing, network reconstruction, i.e. the determination of direct links within the network, is one of the main challenges of systems biology. It is complicated by the fact that local perturbations, such as inhibition of one enzyme, propagate through the network, thereby masking the local, direct interactions. Modular Response Analysis (MRA) facilitates network reconstruction from systematic steady state perturbation data and has been widely applied in the community to infer e.g. signaling networks or gene regulatory networks. MRA implicitly assumes that the network is organized in a modular manner such that communication between modules involves only information transfer, but not transfer of moieties between the modules. The information transfer is facilitated by the modules' outputs, so-called communicating species.
However, interactions such as phosphorylation involve the formation of a complex of kinase and substrate. This complex is linked to both modules via moiety transfer since there is an explicit conversion between free and bound forms of kinase and substrate. As a consequence, the modularity of the network is abrogated and MRA cannot be applied unambiguously. If the concentration of the intermediate complex is not negligibly small, a feedback from the substrate to the kinase is predicted when MRA is applied.
The feedback indicates the sequestration of the active free kinase by the complex and might lead to wrong conclusions about the regulatory wiring of the network. We propose an addition to the standard MRA procedure which

allows to distinguish sequestration connections from regulatory connections. By independently measuring the complexes' responses to the perturbations, it is possible to define a new communicating species as the linear combination of the free enzyme and the complex. The influence of the complex can now be weighted by a free parameter such that the sequestration feedback is eliminated from the local interaction map and the network once again resembles the regulatory wiring.
In a simulation study, we apply the procedure to a number of test models, resembling important network topologies such as the well-known MAPK signaling cascade Raf-MEK-ERK with regulatory feedback from ERK to Raf and from ERK to MEK. While the algorithm still requires some prior knowledge about which of the players act as enzyme and which as substrate, the nature of the inferred feedbacks can now also be determined and a local interaction map free from sequestration feedbacks can be obtained for several cases of network topology such as linear signaling cascades such as the Raf-MEK-ERK cascade.

Keywords: Modular Response Analysis, Sequestration, Feedbacks, Network reconstruction, Modularity, Module, Reverse Engineering

SBMC18-89

Inflammation-dependent suppression of metabolic gene networks in acute and chronic liver disease

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Background & aims: Acute inflammation has been recognized as essential for restorative regeneration. However, whether these processes are interlinked at the level of transcriptional regulatory networks (TRNs) remains unclear.
Methods: Genome-wide expression and TRN analysis were performed in mouse liver after acute injury by CCl4, lipopolysaccharide and tunicamycin exposure, experimental hepatocellular carcinoma (HCC), and human chronic liver disease (non-alcoholic fatty liver, HBV infection and HCC), together with spatio-temp investigation of signalinf and transcription factor expression by western blotting and immunohistochemistry.
Results: The TRNs induced by acute CCl4 intoxication were classified in three core motifs, namely induction of ER stress and inflammation, and suppression of mature liver functions. These motifs occurred within one inevitably interlinked network, starting already 2h after CCl4 administration. Proliferation and tissue restorative TRNs occurred only in a later wave. The interlinked inflammation-metabolism response was also observed in mouse models of chronic liver inflammation and appeared conserved in human liver disease.
Conclusions: Comprehensive bioinformatics identified an interlinked and conserved TNR of upregulated inflammatory and downregulated mature liver functions modules. Restorative regeneration occurs as a subsequent wave that is not part of the initial inflammation-metabolism TRN.

SBMC18-53

Network coherences - a universal approach to quantify the match between 'omics' data and a biological network

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Network-based analyses of 'omics' data are a cornerstone of systems medicine. Their goal is to quantify and statistically evaluate the clustering of biological signals (e.g., co-expression of genes) in a network (e.g., a metabolic network or a protein-interaction network).
Network coherences are topological indices evaluating the connectivity of subnetworks spanned by the 'omics' signal of interest [1,2]. They have been used very successfully to identify scientifically relevant patient subgroups in disease cohorts [2-4].
Here, we aim at a deeper theoretical understanding of network coherence. Using various random walk models on graphs, we test, refine and calibrate this method. In this way, the dependence of a given network coherence upon the number of (e.g., disease-associated) genes, the topology of the underlying biological network or the fragmentation of the functional signal in the network can be studied numerically and compared to analytical predictions.
Our method allows us to detect functional signal even in very noisy data. The main novelty of this approach lies in taking into account collective expression profiles of the whole group of patients and contrast it with individual ones. In order to find relevant signals we "tune" parameters of the collective expression extraction procedure with respect to maximization of the network coherence. This allows us to pick up structures which are not detectable when dealing with individual patients separately.
Based upon our results, we also present a range of applications of (in particular metabolic) network coherence to the

analysis of transcriptome profiles in chronic inflammatory diseases.
This investigation seems to have huge potential for further development and following applications also outside of this specific field.

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Keywords: metabolic network, gene expression, inflamatory disease

SBMC18-40

Quantifying Tissue Mechanics and Tissue Formation with High Resolution Models

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In late stage liver fibrosis or cirrhosis, contrary to healthy tissue, the tissue micro-architecture is largely disturbed and exhibits local areas of compressed cells and cells obstructed by ECM.
Our goal is to study, using mathematical models, the capacity of these cells to migrate and regenerate given the in-situ micro-architecture. Accurate quantification of mechanical stresses and confinement for these cells suggest models that can represent cell shape and estimate the forces on the cells at high resolution.
We present a novel "Agent-Based Model" in which cells are represented individually and constructed by a network of viscoelastic springs that permits every cell to deform in a realistic way. The model allows to mimic cell migration, growth, division and death. We call this model type "Deformable Cell Model" (DCM). This approach is opposed to popular so-called "center-based" models (CBM) in which cells are represented by a rigid geometry and cannot mimic cell shapes that are adapted to the environment.
The mechanical parameters in our model are physically tangible and can be calibrated using single cell optical stretcher experiments. In a next step we investigate the influence of the cell compression level on the growth rate of cells, by validation of simulations of multi-cellular spheroids growing in a calibrated elastic capsule with experimental data. We find that the individual cell growth rate decreases non-linearly with compressive strain, characterized by a specific mathematical function.
As a test case for our model, we have performed simulations of regenerating liver lobules after CCL4 intoxication where hepatocytes are migrating towards the central vein. We have considered both CBM and DCM (see Fig. 1).

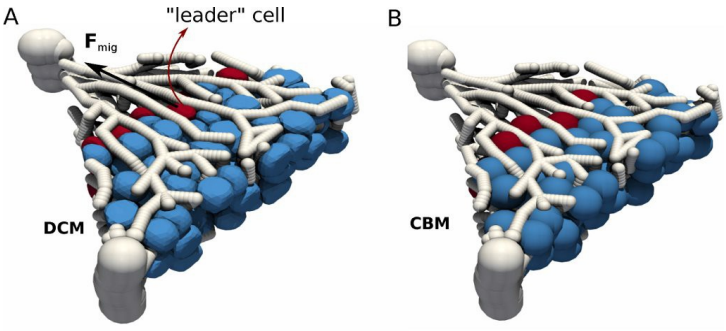

Fig. 1 Snapshot of a regenerating part of a liver lobule simulation with left: high resolution Deformable Cell Model (DCM), and right: center-based model (CBM). The white structures represent the bloodvessel network.

From our simulations we conclude that migrative forces exerted by the cells on the ECM scaffold in the lobules, potentially needed to squeeze through the bloodvessel network and finally close the lesion in a certain time, may be be overestimated if a model with a non-adapting cell shape (CBM) is used. Simulations with DCM require significant lower migration forces.
Overall, this study shows that the capability of a model to correctly capture the interplay of cell environment, mechanics

and cell shape could be of crucial importance to understand cell behavior and cell fate in liver fibrosis or cirrhosis.

Keywords: High resolution mathematical model, Cell mechanics, Tissue regeneration, Liver

SBMC18-46

Quantitative analysis of 3D geometrical models of human liver tissue affected by non-alcoholic Fatty Liver Disease (NAFLD)

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Liver tissue shows a unique and complex three-dimensional (3D) tissue architecture. It consists of two intertwined and intricate networks, the sinusoidal network for blood flow and the bile canalicular network for bile secretion. These two networks have an intrinsic three-dimensional topology and therefore, their structure cannot be deduced from two dimensional (2D) approaches. Yet, the gold standard to diagnose liver diseases, such as non-alcoholic Fatty Liver Disease (NAFLD), is based on the analysis of 2D histological images. To improve our understanding of liver physiology and physiopathology, we developed a pipeline for the 3D reconstruction of liver tissue sections obtained by biopsies from healthy individuals and patients with NAFLD at different stages of disease progression. The main components of liver tissue architecture were reconstructed and analysed, including central and portal veins, bile canaliculi, sinusoids, hepatocytes, as well as subcellular structures such as nuclei and lipid droplets. The resulting tissue reconstructions provide a complete description of different morphological parameters across multiple scales, from tissue to subcellular level, and uncovered a significant amount of new information about morphological changes occurring in NAFLD. Further work will focus on identifying statistically relevant biomarkers, e.g. hepatocyte ploidy levels, zonated lipid droplets agglomeration, etc., predictive for the early detection of disease in human liver samples in NAFLD aimed at gaining new insights into its pathogenic mechanism.

Keywords: Non-alcoholic Fatty Liver Disease, Tissue reconstruction, Quantitative analysis, 3D

SBMC18-63

Use of deep learning methods to translate drug-induced gene expression changes from rat to Human hepatocytes

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Introduction: In clinical trials animal and cell line models are often used to evaluate the toxicity of a novel or existent chemical. However, relating the results of animal and in vitro model exposures to the human in vivo state presents a challenge. The repeated dose rat bioassay has been shown to lack sufficient sensitivity and specificity in terms of predicting toxic effects of pharmaceuticals in humans while in vitro models lack the interplay with other tissues. In this study we propose to use deep learning techniques to predict time series of human in vitro gene expression from rat in vitro gene expression following exposure to several compounds. We will also apply the developed method to relate in vivo gene expression to in vitro expression following an exposure.
Materials & Methods: The TG-GATES data set is a toxicogenomics database containing gene expression profiles from in vivo rat and in vitro primary rat and human hepatocytes following exposure to 170 compounds at multiple time points and dosages (low, medium, and high) [1]. For this study we use a subset of 47 compounds for which all time points and dosages are available in all three domains. (rat in vivo, human and rat in vitro). Using replicates and controls, 754 learning examples can be generated, of which 80% are used for training and the remaining 20% reserved for testing. Given the relatively limited number of learning examples we identified a subset of 85 genes selected to allow classification of compounds based on toxicity.
Results: Multiple network architectures were utilised and compared including convolutional neural networks and capsule networks, the latter also encodes feature position [2]. More traditional methods of encoding the data were implemented in order to extend the number of genes that could be included in the analysis. Unsupervised domain adaptation has also been employed to predict human in vivo gene expression for which we currently have no data [4].
Conclusion: Our study evaluates the ability of artificial neural networks to predict both human in vitro and rat in vivo

gene expression following exposure to a compound given gene expression of exposed primary rat hepatocytes in culture. In future work we aim to utilise our models to predict human in vivo gene expression, permitting the evaluation of toxicity of a compound on predicted human in vivo gene expression rather than the direct inference of toxicity from exposure to an animal or cell line model. In addition, cursory exploration of the latent spaces generated in these neural networks suggest a promising new method for the classification of a compound's toxicity.

References:

[1] Igarashi Y et al. "Open TGGATEs: a large-scale toxicogenomics database", Nucleic Acids Res, 2015 Jan;43(Database issue):D921-7
[2] Hinton GE, Sabour S, Frosst N. "Dynamic Routing Between Capsules" ,2017 arXiv:1710.09829
[3] Ganin Y et al. "Domain-adversarial training of neural networks", J Mach Learn Res 2016;17:2096-2030.

Keywords: Machine Learning, Artificial neural networks, Gene Expression, Toxicogenomics

SBMC18-73

Substrate-based Kinase Activity Inference: interpreting phosphoproteomic data using computational enrichment analysis

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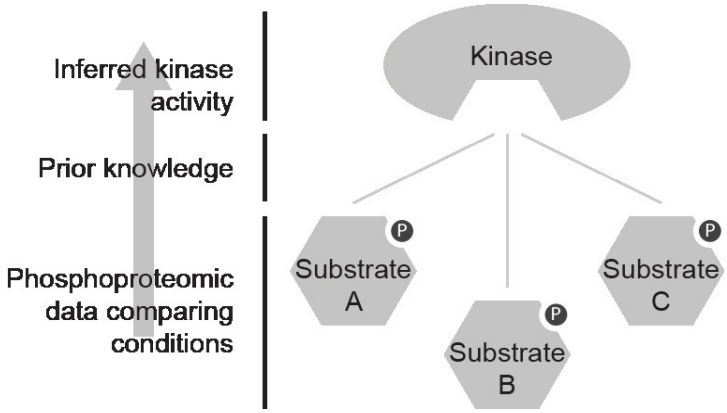
Phosphoproteomic data generated by global mass spectrometry (MS) contain high-content information on protein phosphorylation, a process central to cellular signaling. Nevertheless, the biological function of the vast majority of phosphorylation sites remains unknown. This presents a challenge in drawing broad biological conclusions from phosphoproteomic data. We have developed Substrate-based Kinase Activity Inference (SKAI), a methodology to infer kinase activity from phosphoproteomic data. We draw upon prior knowledge of kinase-substrate interactions to construct custom lists of kinases and their respective substrate sites, termed kinase-substrate sets. Using these sets within the Gene Set Enrichment Analysis (GSEA) framework, we applied our kinase activity inference methodology to global phosphoproteomic data from two mouse models of inflammatory bowel disease (IBD). SKAI results have been validated for MK2 here and for PAK in the literature. Although SKAI identified multiple dysregulated kinases in each of these two mouse models, only GSK3B was common to both. This example acts as an illustration of the potential utility of SKAI in elucidating new drug target leads for the treatment of complex disease.



Workflow diagram illustrating bottom-up approach to infer kinase activity from prior knowledge and phosphoproteomic data.

Keywords: Phosphoproteome, Inflammation, Kinases, Computational Biology, Target identification

SBMC18-85

A computational physiology-based bile acid model

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Drug-induced liver injuries (DILI) are an important issue in drug development and patient safety and often lead to termination of drug-development programs or late withdrawals of drugs. Since DILI events are often hard to diagnose in preclinical settings, a need for alternative prediction methods such as computational modeling emerges. Impairment of bile acid (BA) metabolism, known as cholestasis, is a frequent form of DILI. Being rather a systemic then a single organ related disease,
whole-body physiology-based (PB) modeling is a predestined approach for cholestasis modeling. Our objectives are 1) the development of a physiology-based model for the human bile acid metabolism, 2) model validation and characterization for a virtual population, and 3) prediction and quantification of genetic predispositions on bile acid metabolism.
The developed bile acid model (BAM) is based on a reference PB model implemented in PK-Sim as part of the Open Systems Pharmacology Suite (OSPS), version 7.2 and describes the bile acid circulation in a standard male individual. Active processes such as the hepatic synthesis, gallbladder emptying upon meal intake, transition via the gastrointestinal tract, resorption into the liver, distribution within the body, and excretion are included. The BAM allows simulations of BA exposure in relevant tissues such as the liver and therefore enhances the mechanistic understanding of cholestasis. The kinetics of active processes for the surrogate BA glycochenodeoxycholic acid were fitted to time-concentration profiles of blood BA levels reported in literature. The robustness of our BAM is underlined by simulating the plasma BA concentrations for a virtual population of 1,000 individuals. In addition, disease phenotypes as Benign Recurrent Intrahepatic Cholestasis (BRIC) type 2 can be simulated. Simulations of our PB BAM suggest a higher susceptibility of BRIC patients towards cholestatic DILI, which is congruent with literature. Apart from these intrinsic effects, drug-interactions and their effect on the systemic bile acid metabolism can be simulated by combining the PB BAM with drug PBPK models or in vitro omics data. Altogether, the presented model enhances our mechanistic understanding of cholestasis, allows the identification of drug-interactions leading to altered BA level in blood and organs, and could be used to prevent clinical cases of cholestasis and enhance patient safety.

Keywords: PBPK modeling, bile acids, computational, disease model

SBMC18-12

Efficient parameterization of large-scale dynamic models using relative protein, phospho-protein and proliferation measurements

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Mechanistic models of signaling pathways provide the means to integrate heterogeneous data and to gain a quantitative understanding of cellular physiology. However, many models only focus on individual pathways, neglecting many others and ignoring any cross talk. Larger models on the other hand come with big challenges for parameter estimation, not only in the form of high computational demand. In particular, we found that relative measurements - which is what most large-scale datasets are - drastically impair optimizer performance.
In this study, we consider a large-scale ordinary differential equation model of cancer-related signaling (>4000 kinetic parameters, >1000 state variables) and parameterize it using relative measurements of protein, phospho-protein and proliferation data from cancer cell lines. We demonstrate the loss of information due to relative data and the associated decline in optimization robustness with different types of optimizers. We subsequently demonstrate how a novel hierarchical optimization approach in combination with adjoint sensitivity analysis can be applied to recover optimizer performance and parameterize large models. We show how our approach allows computing proportionality factors, offset parameters and error model parameters analytically, thereby simplifying the numerical optimization problem considerably and rendering it solvable by previously failing optimizers. Furthermore, we show that this approach allows us to estimate error model parameters with negligible computational overhead when no experimental estimates are available. The estimated distribution of measurement errors provides unbiased means to weight heterogeneous datasets. Finally, we show that integrating molecular data with phenotypical data improves model generalization when predicting drug response phenotypes of cancer cell lines.
Overall, our hierarchical optimization approach allows for the efficient parameterization of large-scale dynamic models based on heterogeneous relative measurements and can easily be adopted by other researchers.

Keywords: computational biology, parameter estimation, large-scale dynamical models, optimization, data integration

SBMC18-27

Time-harmonic ultrasound elastography to assess pediatric non-alcoholic fatty liver disease

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Overview & Aims: Non-alcoholic fatty liver disease (NAFLD) is the most frequent chronic liver disease in children and adolescents and may present with simple steatosis or progressive liver fibrosis. While liver biopsy remains the gold standard to determine disease activity, non-invasive means to determine structural changes of the liver are warranted. The aim of this study was to apply novel ultrasound time-harmonic elastography (THE) in a cohort of pediatric patients with biopsy-proven NAFLD and determine its diagnostic utility to distinguish different stages of fibrosis.
Methods: THE was performed in 67 consecutive pediatric NAFLD patients (age range 10-17 years; mean BMI 34.7 kg/m2; range, 21.4-50.4 kg/m2) and AUROC values for the detection of any (F>0), moderate (F>1) or advanced (F>2) fibrosis were calculated to assess its diagnostic performance. The best liver stiffness (LS) cut-offs for the detection of each fibrosis stage were determined by using Youden-Index.
Results: AUROC values for the detection of any, moderate or advanced fibrosis were 0.88 (95% CI: 0.80-0.96), 0.99 (95% CI: 0.98-1.00), and 0.88 (95% CI: 0.80-0.96). The best LS cutoffs were 1.52 m/s for F>0, 1.62 m/s for F>1, and 1.64 m/s for F>2. *Conclusion*: THE precisely detects moderate fibrosis in pediatric patients with NAFLD, while it is less accurate in the detection of any or advanced fibrosis. Further studies in independent cohorts are warranted to validate its diagnostic utility for non-invasive assessment of fibrosis in pediatric NAFLD.

Keywords: NAFLD; pediatrics; Non-invasive diagnostics; elastography

SBMC18-81

Mathematical modelling of liver fibrosis patterns: preliminary models

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Fibrosis is a consequence of repetitive liver injuries, e.g. upon viral infection, alcohol consumption, male nutrition or hepatotoxicants. Based on the etiological factor, liver fibrosis develops in different patterns and presents as septal in toxic injuries, biliary in cholestatic diseases, bridging upon hepatitis virus infections or pericellular in case of alcohol consumption. The mechanism behind the generation of the different patterns is elusive. Computational modeling of liver fibrosis pattern formation may contribute to understanding this mechanism. According to time-resolved experimental data from carbon tetrachloride (CCl4) treated mice, the distributed CYP2E1-positive hepatocytes in the liver would gradually form bridging pattern connecting the central veins. Such bridging pattern was also observed for fibrotic collagens in the later stage. This similarity suggested that the spatial pattern of CYP2E1 might indicate the location where the fibrosis forms. Here we developed a dynamic activator-inhibitor system to study liver fibrosis formation, where the activator is a diffusible protein released from the central vein to promote the CYP2E1 signaling while the inhibitor is a diffusible protein released from the portal vein to inhibit the CYP2E1 signaling. Currently, this model can partially capture the observed patterns of CYP2E1 and extracellular matrix (ECM) upon chronic liver injuries.
In addition to this CYP2E1-dependent fibrosis formation assumption, we are currently tuning our model by adding more assumptions independent of CYP2E1 signaling. These assumptions include, but is not limited to i) crosstalk between activated hepatic stellate cells and liver sinusoidal endothelial cell differentiation; ii) The dialogue between endothelial cells lining the hepatic veins and hepatocyte metabolic zonation; iii) Presence of so far unknown diffusible inhibitor in the portal compartment, i.e. a bile duct driven factor.

Keywords: Liver fibrosis, CCl4 mouse model, Mathematical model

Systems Medicine & Clinical Applications

SBMC18-84

Senile Abcb4-/- mice upon acute toxic insult recapitulate features of acute-on-chronic liver failure in patients

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Acute-on-chronic liver failure (ACLF), characterized by acute decompensation and multi-organ failures, is a recently recognized disease entity with poor outcome (short-term mortality rate 30-40%). Several histological features were identified in ACLF patients, e.g. submassive cellular necrosis and ductular reactions. Understanding mechanisms controlling organ deterioration or recovery is hampered by lacking a suitable animal model. The current study investigated pathological alterations in 75 weeks old Abcb4-/- mice exposed to 3.2 g/kg carbon tetrachloride (CCl4) intraperitoneally. Liver necrosis and regeneration were investigated in a time-resolved manner. Survival analysis revealed that 33% of mice died during the first 24h after CCl4.
Mice recovered if they passed this critical period. Histologically, massive hepatic necrosis was recorded at days 1 and 2 after CCl4, which recovered at day 4. Proliferation index was measured by Ki-67+ nuclei, illustrating massive proliferation at days 2 and 4 following CCl4 injection. Hepatocyte-cholangiocyte cell plasticity was investigated by CK19 immunostaing, indicating that generation of numerous CK19+ hepatocytes starts immediately after acute insult. Molecular phenotyping of mouse livers and comparative transcriptomics analysis is currently ongoing. In conclusion, administration of a high dose of CCl4 on top of
genetically manipulated cirrhosed livers induces a phenotype that recapitulates at least some ACLF patient features. These are (1) a golden window, (2) cirrhosis, (3) ductular reaction, (4) submassive hepatic necrosis and (5) hepatocyte plasticity.

Keywords: ACLF, Abcb4-/-

SBMC18-98

Physiologically-based pharmacokinetic modeling for the analysis of drug pharmacokinetics in populations with impaired liver function

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[2]*Dr. Margarete Fischer-Bosch Institute (Stuttgart)*
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Physiologically-based pharmacokinetics (PBPK) modeling is a mechanistic modeling technique that describes the absorption, distribution, metabolism and excretion of xenobiotics in an organism at a high level of physiological detail. PBPK models incorporate physiological information on the whole body and contain representations of all important organs, e.g. the liver. The liver is the main site of drug metabolism and, therefore, plays an essential role in the pharmacokinetics of many drugs. Functional impairment of the liver, as it is the case in diseases like for example liver cirrhosis, can impact drug pharmacokinetics and, thus, may require dose adjustment. PBPK modeling is an ideal technique for the analysis and simulation of pharmacokinetics in patient populations with specific pathophysiology because of its mechanistic model structure. The objective of the presented work was to evaluate the potential and critical gaps of current concepts in PBPK modeling and its predictive power for an assessment of liver cirrhosis.
In a stepwise approach, the current state of knowledge on physiological changes related to liver cirrhosis were compiled and integrated into a PBPK framework. In this proof of concept study, physiological changes considered in the PBPK models such as blood flows, plasma protein concentrations, hematocrit, liver enzyme activities and glomerular filtration rate (GFR) were adjusted according to literature values [1]. By this, in silico hepatic impairment (Child-Pugh A/B/C) populations were established and population simulations with three paradigm compounds were performed to test the predictive accuracy and structural relevance of the compiled information.
The modified PBPK models were able to predict the mean pharmacokinetics of all three paradigm compounds under hepatic impairment well regarding the time profiles as well as parameters like the maximal concentration (Cmax) and the area under the curve (AUC). Nevertheless, the approach struggled with the simulation of the right variability within and between populations. Further analysis identified different underlying reasons, one of them being the limited translatability of the classification rules into physiological parameters, as well as the pathophysiological heterogeneity of populations with the same classification score.
In a next step, the identified gaps and problems will be addressed by further literature research with special attention given

to the correlation between classification rules and altering effect on pharmacokinetics as well as to the integration of co-morbidities into the model framework. Finally, the concepts developed will be applied to new clinical pharmacokinetics data, for which patients are treated with a cocktail of six marketed drugs in order to measure hepatic drug clearance capacity in vivo.

Reference:
[1] Edginton AN et al. Clin Pharmacokinet. 2008; 47(11):743-52.

Keywords: Physiologically-based pharmacokinetic modeling, Systems pharmacology, Liver cirrhosis, Hepatic impairment

SBMC18-60

CD271 antibody-labeled magnetic beads for isolation of murine hepatic stellate cells

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Hepatic stellate cells (HSCs) are the main extracellular matrix (ECM) producer cell and play crucial roles in liver regeneration, immune regulation, and fibrosis. The current HSC isolation methods are mainly based on their low density caused by the intracellular storage of fat droplets that consist mostly of retinoid-palmitate. Therefore, HSCs phenotype i.e. quiescent (more lipid) and activated (less lipid) is affecting the yield. Nerve growth factor receptor (NGFR or CD271) is mesenchymal stem marker and expressed on the surface of HSCs in human, rats, and mice. Here, we tested CD271 antibody-labeled magnetic beads (Miltenyi Biotec, Catalogue Nr.: 130-091-885 and 130-048-801) for HSC purification and their efficacy were examined with respect to cell numbers, purity, phenotype, and reproducibility. Livers of adult Balb/C or C57bl/6N wildtype mice were washed in situ with EGTA buffer and digested with collagenase. Firstly, hepatocytes were isolated by low-speed centrifugation followed by a percoll-based purification step. We then applied CD271 antibody-labeled magnetic microbeads on the remaining cell suspension to isolate HSCs using the magnetic cell sorting (MACs) technology. For comparison, HSCs were also isolated according to the standard procedure with a Nycodenz gradient. The purity of the HSCs preparation was investigated by co-immunofluorescence, immunoblot, qPCR, and FACs. CD271-"enriched" cells show UV fluorescence after stimulation with UV light and display co-staining with DESMIN, a classic marker for quiescent and activated HSCs. This was further confirmed by FACs and immunoblot analysis, revealing that CD271 enriched cells are predominantly DESMIN positive, and F4/80 (Kupffer cells) and CD31 (endothelial cells) negative. Further, CD271 mRNA was enriched in purified HSCs and could not be detected or only at very low levels in other cell types.
In conclusion, CD271 is a specific HSCs marker in mice, which can be exploited for their purification. The CD271 microbeads-based procedure for HSCs isolation represents a cost-effective, well-reproducible and flexible method when compared with current density-gradient-based isolation protocols.

Keywords: CD271, hepatic stellate cell

SBMC18-67

Application of physiologically-based pharmacokinetic modeling for the characterization of a murine liver disease model and its relevancy for cross-species extrapolation

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Physiologically-based pharmacokinetic (PBPK) models are mechanistic mathematical models that describe administration, distribution, metabolism and excretion of xenobiotics within the body of an organism. Organs are represented by compartments which are interconnected by the blood flow and which are further divided in subcompartments such as the cellular space, the interstitium, red blood cells and plasma. In addition to physiological parameters, compound-specific parameters are required to calculate for example organ-plasma partitioning and passive tissue permeation such that drug concentration in tissue can be simulated as a function of time.
PBPK modeling becomes increasingly important in drug development starting from prediction of safe doses for the first-in-man study until the simulation of in silico trials of pharmacokinetics in patients including specific patient cohorts for example with impaired liver function. In all phases of pharmaceutical development, PBPK modeling can assist clinical study planning, inform dosing regimes and predict drug exposure that can be related to the effect.

The liver is the central organ in drug metabolism and the consideration of the contribution of hepatic clearance to total elimination is crucial for understanding pharmacokinetics of a compound. Here we present a PBPK modeling study for the comparative characterization of the pharmacokinetics of a drug cocktail in a mouse model for liver cirrhosis as well as human cirrhotic patients. Animal models provide invasive access to disease processes that cannot be studied in humans due to ethical and experimental constraints. However, it is important to determine to what extent animal models can actually contribute to the planning of clinical studies.
In the cirrhosis mouse model, toxic liver damage was induced by administration of carbon tetrachloride (CCl4) over the course of 12 months. The resulting fibrosis of the hepatic tissue causes changes in the clearance capacity of the liver. A drug cocktail of torsemide, pravastatin, codeine, caffeine, midazolame and talinolol was administered to estimate the remaining clearance capacity of the liver after 2, 6 and 12 months of CCl4 exposure.
Following a previously established workflow (Schenk et al., 2017) PBPK modeling was used to quantify the functional change in hepatic clearance capacity. The models will be further refined by incorporation of information from experimental analyses at the tissue scale and the cellular level. It is planned to systematically compare the pathophysiological changes in the mouse model to the ones observed in human patients to improve relevance of animal testings for cross-species extrapolation in the future.

Reference:
Schenk A, Ghallab A, Hofmann U, Hassan R, Schwarz M, Schuppert A, et al. Physiologically-based modelling in mice suggests an aggravated loss of clearance capacity after toxic liver damage. Sci Rep. 2017;7(1):6224.

Keywords: PBPK modeling, Systems Pharmacology, liver cirrhosis, cross-species extrapolation

SBMC18-6

Integration of Representative Sinusoids into a Physiologically Based Whole-Body Model for a Detailed Description of Biliary Transport

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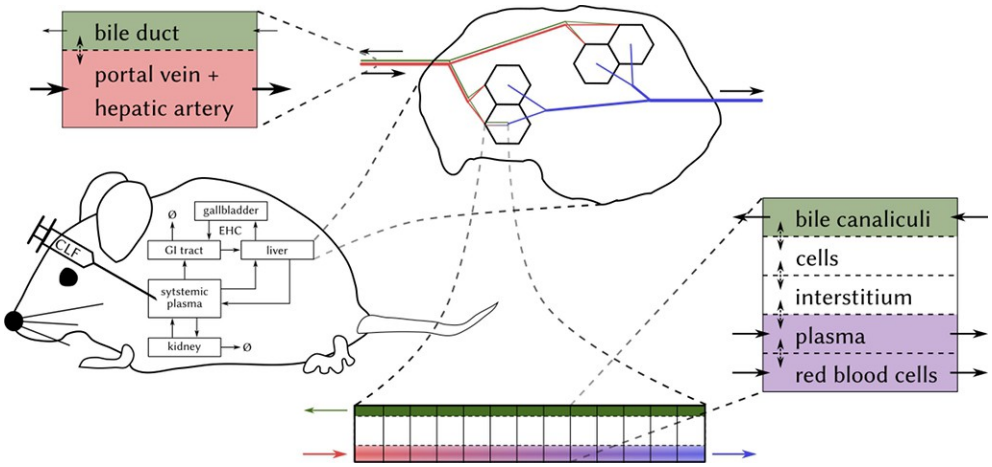
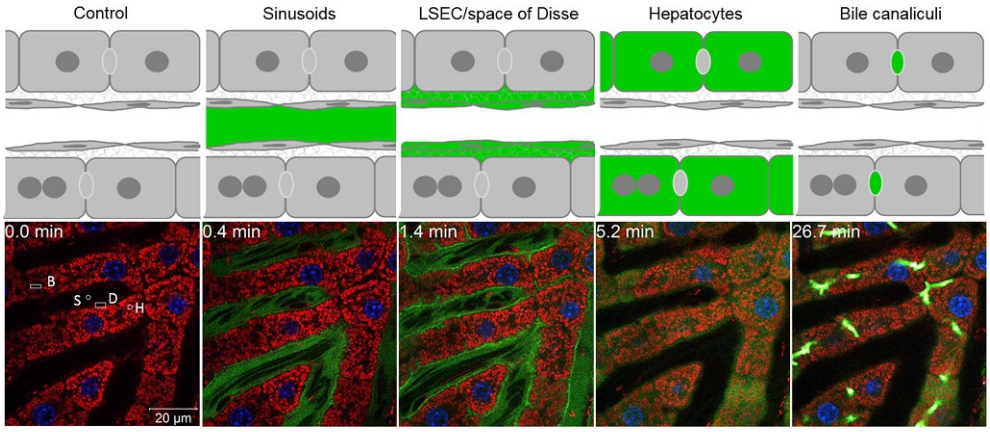
Enterohepatic circulation (EHC) plays an important role in the distribution and clearance of many drugs. However, the kinetics of the underlying transport processes are unkown to date. Using cholyl-lysyl-fluorescein (CLF, an analogon of natural bile acids) as a probe molecule, we here quantify the sequence of steps involved in EHC, i.e., uptake into hepatocytes, secretion to bile canaliculi, transport via bile flow to the gallbladder and the gastrointestinal (GI) tract and ultimately re-absorption from the duodenum.
Physiologically based pharmacokinetics (PBPK) models are well suited to describe EHC of molecules since these models implicitly describe the systemic interplay of several organs at whole-body level. However, PBPK models usually neglect the physiology of bile flow and rather represent the EHC of compounds as a functional process without any real level of physological detail.
Well-stirred PBPK models can be used if hepatic zonation or heterogeneity of perfusion and metabolic processes can be neglected. Spatial heterogeneity can be alternatively considered by introducing spatio-temp resolution in extended liver models, e.g., using the four-scale "representative sinusoid" approach [DOI 10.1371/journal.pone.0133653]. Still, state-of-the-art PBPK models have limited capabilities of representing compound transport through bile flow due to its spatio-temp complexity.



Sketch of the model structure for extending the "representative sinusoid" framework by biliary transport

We here present a structural extension of the "representative sinusoids" concept to also account for bile canaliculi and the transport to the gallbladder and the GI tract, for the use case of the bile acid analogon CLF. The systemic distribution of this compound in the liver, the kidney and the GI tract has recently been measured by in-vivo, two-photon microscopy-based imaging [DOI 10.1007/s00204-016-1906-5]. This analysis allowed quantifying transport and elimination of CLF in vivo, identifying important input parameters in whole-body PBPK models.



In-vivo imaging of CLF in the liver, adapted from Fig. 4 in [DOI 10.1007/s00204-016-1906-5] (CC-BY licensed)

Ultimately, the goal of our study is to represent the EHC of bile acids at the whole-body level including a physiologically detailed liver model.

Keywords: enterohepatic circulation, physiologically based pharmacokinetics models, cholyl-lysyl-fluorescein, multiscale modeling, bile flow

SBMC18-56

The influence of smoking on the CYP1A2-dependent dynamic liver function test

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Background: Targeting tissue-specific expressed cytochrome P450 (CYP) isoforms enables the quantification of a variety of functional properties. The exclusive metabolism of methacethin by hepatic CYP1A2 allows the determination of hepatic functional biotransformation capacity and can be used as surrogate parameters for liver disease grading. While genetic polymorphisms only play a minor role, several drugs and lifestyle factors, such as smoking or caffeine consumption, were shown to influence heptic CYP1A2 expression and activity. Understanding the impact of influencing factors on the test results is required for more precise test result interpretation.
Methods: In a retrospective clinical database analysis, we identified 730 patients with liver pathologies (cirrhosis or HCC) who underwent a LiMAx test and reported smoking habits. We compared LiMAx values between active smokers and non-smokers and analysed the effects of reported smoking on the day of, or the day before the LiMAx test or smoking habits further back in the past. A physiological based pharmacokinetics model of the LiMAx test was used to analyse the effect of smoking on the test results.
Results: Amongst all analysed patients with liver disease, the LiMAx test result was significantly higher in those reporting to be active smokers, as compared to non-smokers (mean±SD 375±211 vs. 305±149 µg/kg/h, $p<0.05$). No significant differences were found between the duration of smoking history (pack years) and the LiMAx values, while significant ($p<0.05$) differences were found between patients smoking on the day of the LiMAx test and those who smoked on the day before the test, but not on the day of the test (347±229 vs. 383±206). A dose-response relationship was found between the number of cigarettes smoked on the test day before the test and the test outcome in cirrhotic patients. The mean (±SD) LiMAx values increased from 153 µg/kg/h (±129 µg/kg/h) in non-smoking cirrhotic patients to 208 µg/kg/h (±162 µg/kg/h) in cirrhotic patients who smoked 1-5 cigarettes and 229 µg/kg/h (±144 µg/kg/h) in patients who smoked more than 6 cigarettes. The computational model correctly predicted the observed changes of LiMAx in active smokers and allowed to estimate the effects of CYP1A2 changes on test results.
Conclusion: Collectively, our data indicate that smoking has a short-time effect on the CYP1A2-dependent LiMAx test outcome. Integration of the clinical data into a mathematic model allows precise prediction of the smoking-induced CYP1A2 alterations and could potentially be applied for improved test data interpretation in smoking patients.

Keywords: dynamic liver function test, CYP1A2, smoking

SBMC18-90

Improved evaluation of caffeine-based dynamical liver function tests by accounting for lifestyle and pharmacological modifiers like smoking and contraceptive use

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Quantitative dynamical liver function tests evaluate the function of the liver via the clearance of a given test substance, thereby providing in vivo information on the metabolic capacity of the liver. The liver function test based on caffeine is known for long, but its clinical usability is hampered by large interindividual variability and dose-dependency. In this work we developed a detailed physiologically based pharmacokinetics model (PBPK) for the evaluation of the caffeine clearance test, assessing hepatic conversion of caffeine to paraxanthine via cytochrome P450 CYP1A2. The model is able to reproduce results from a wide range of reported studies under varying caffeine doses and application routes. The model accounts for interindividual differences based on distributions of CYP1A2 and modification of CYP1A2 activity via lifestyle factors, e.g. smoking, and pharmacological interactions,
e.g. contraceptives. Validation was performed with an independent clinical trial (EudraCT 2011-002291-16, ClinicalTrials.gov NCT01788254) demonstrating an improved prediction using individualized models accounting for smoking status and contraceptive use. Hereby, we could reduce the large variability in the test results providing the basis for better sensitivity and specificity in diagnosing subjects with liver problems.

Figure 1. Stratified and individualized pharmacokinetics models. A) Clinical protocol for caffeine-based liver function tests. B) Overview over physiological based pharmacokinetics model for caffeine (and caffeine tests). C) Mean pharmacokinetic model performs poorly when simulating stratified data sets. Subgroups are smokers (smoker), contraceptive users (cs), smoking contraceptive users (cs-smokers) or non-smokers not taking any contraceptives (control). D) Including lifestyle factors by shifting the underlying CYP1A2 distributions. E) Resulting stratified model predictions in much better agreement with clinical data. F) Stratified model predictions over a wide range of caffeine doses are in good agreement with pharmacological parameters from multiple studies. F) Body weight adjusted model predictions. G) Individualized model predictions using smoking and contraceptive information in combination with body weight. Such individualized pharmacokinetics models outperform the classical approach of body weight adjusted models in the prediction of pharmacokinetic properties of caffeine. All data from EudraCT 2011-002291-16, ClinicalTrials.gov NCT01788254 (labeled IKP243 in figure).

Keywords: caffeine, paraxanthine, liver function test, CYP1A2, smoking, contraceptives

SBMC18-8

Growth Factor Signaling in Breast Cancer - a Dynamic Model of the MAP Kinase Pathway

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Targeted therapies have shown striking success in the treatment of cancer over the last years. However, their specific effect on the tumor seems to be varying and difficult to predict. Using an approach of mathematical modeling based on ordinary differential equations we are able to gain insights into the cellular response of breast cancer cells to the treatment with several monoclonal antibodies.
The MAP kinase (MAPK) cascade, a major signaling module responsible for cell growth, represents the core of the developed mathematical model. Describing the activation dynamics of three main players in this signaling pathway, RAF, MEK and ERK, it serves as intracellular readout of growth factor stimulation. The varying response of cells to growth factors as well as to the treatment with breast cancer specific drugs strongly depends on the abundances and compositions of receptors in different breast cancer types. This link between the intracellular signaling response and the first contact point of growth factors with a cell, the cell surface receptors, is generated by a mechanistic input module. The input module translates receptor data of a given cell type into specific dimerization and phosphorylation patterns, which finally activate the downstream signaling cascade. Information about
the mode of action of specific drugs, mostly targeting growth factor receptors, is integrated in this mechanistic module.
The model calibration is performed using time-resolved measurements of differentially phosphorylated forms of involved proteins. Experimental data are generated via reverse phase protein arrays for five breast cancer cell lines with varying receptor compositions and abundances. Finally, the goal of the model is to predict the response of the system to different drug treatments for specific breast cancer cell lines. In clinics, the receptor properties of tumor cells can be easily determined along with the analysis of the cancer type. Thus, dynamic modeling of signaling pathways could open a door for personalized medicine in cancer treatment.

Keywords: Mathematical model, Ordinary differential equation, Mitogen-Activated Protein Kinase, Breast cancer, Signal transduction, Growth factor

Other

SBMC18-47

Bile acid toxicity: Tissue level adaptations and intracellular mechanism in cholestatic liver disease

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Cholestasis is a commonly occurring pathological condition in the liver that results in hepatotoxicity. It results in accumulation of bile acids in large concentration in the liver tissue and the bile ducts. Alterations in the bile ducts commonly termed as 'ductular reactions' are a response of the biliary conduits to cholestatic liver injury [1]. We have previously described a detailed 3D response of the IBDs during BDL induced cholestasis. IBDs undergo architectural changes such as lengthening, ramification and extensive enlargement without an increase in their diameter or canalicular connectivity to alleviate cholestasis [2].
Although we have investigated the tissue level adaptations of the liver to cholestasis, the exact intracellular mechanism of bile acid-induced toxicity during cholestasis and its link to such tissue level adaptation are still unclear. To determine toxic concentrations of bile acids to hepatocytes, we utilized time-lapse imaging and cell viability based on the metabolic activity. Our results show that CDCA, a known FXR ligand, is the most cytotoxic bile acid with an EC50 of 100-250 µM, with other important bile acids such as DCA, UDCA, TCA being progressively less toxic. Based on the observations of cell death under bile salt intoxication, we hypothesized that bile acid toxicity is either a result of direct interaction of bile acids with the actin cytoskeletal components or is indirectly affecting the regulation of intracellular FXR signaling to bile acids. We, therefore, developed methods to visualize the two major components of the cytoskeleton: the actin network and the tubulin network, in live primary hepatocytes and HepG2 without the need for or permeabilization and ectopic expression. In HepG2s, these were combined with live cell imaging of an FXR-derived FRET sensor for bile acids to evaluate the propensity of these bile acids to activate FXR signaling.
Using these methods, we could observe alterations of the actin cytoskeleton, specifically of the peri-canalicular cortical actin, eventually leading to canalicular rupture and consequent cell death. Attempts to derive correlations with FXR activity showed that while FXR is active at the concentrations when bile acids are cytotoxic, there was no direct correlation between FXR activity and cytoskeletal perturbation. The investigation of upstream factors that lead to the cytoskeletal disruption upon bile acid exposure is currently underway. The methods of live cell cytoskeletal visualization are also optically compatible with intravital 2-photon imaging and enable the investigation of cholestatic toxicity in mouse models. The technical and biological challenges tackled in this work will eventually help us to zero-in on the exact intracellular mechanism of liver damage induced by bile acids in cholestasis and investigate clinically relevant strategies to resolve cholestatic liver injury.

Keywords: IBDs- interlobular bile duct, BDL-bile duct ligation, FXR- farnesoid X receptor, CDCA- chenodeoxycholic acid, DCA- deoxycholic acid, UDCA- ursodeoxycholic acid, TCA- taurocholic acid

SBMC18-74

Bile Flux Analysis in the Liver Biliary Network

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Bile salts synthesized or absorbed from the blood stream by hepatocytes are secreted into bile canaliculi. Bile canaliculi, formed from the apical membrane of hepatocytes represent a conduit network that connects to the bile duct network formed from cholangiocytes. The biophysical mechanism for the movement of bile salts through the biliary network, and eventually into the
gall bladder is of importance for the two reasons - 1. Interruption of bile flux in the biliary tree, and consequent bile acid accumulation results in cholestatic liver disease due to bile acid toxicity, and 2. The path followed by bile acids is the same as that for pharmaceuticals, and toxins that are excreted through bile. The driving force behind bile flux from canaliculi to the bile ducts has been postulated to be negative osmotic pressure in the biliary conduits due to the high concentration of solutes in bile. Such a negative osmotic pressure is expected to generate convective flow of biliary fluid, which transports bile acids. Hepatocyte contractility is considered a minor contributing factor to this convective flow.
We employed 2-photon and confocal imaging, raster image correlation spectroscopy and in vivo photoactivation, coupled with biological perturbation to investigate the mechanism of bile flux. We discovered that contrary to the above postulates, bile flux in the canalicular network is largely due to diffusion of bile salts from high concentration generated there by hepatocytes. Experiments with secretin-induced bile excretion show that fluid output at the end of the biliary tree (gall bladder) is rather generated by cholangiocytes, and not by hepatocytes. We determine the diffusion coefficient of a bile salt analog in the canalicular network to be ~2 µm^2/s, while convective flow velocity is less than 0.02 µm/s. Internal controls, where blood flow in sinusoids and diffusion in hepatocyte cytoplasm can be measured simulataneously in the same

experiment confirm that in vivo RICS is capable of detecting flow and diffusion (65µm/s blood velocity, ~2µm^2/s diffusion coeff.) and bile canalicular fluid flow is insignificant compared to bile acid diffusion.
We confirm these findings using a photo-activatable analog and demonstrate symmetric dispersion of bile salts through the canalicular network, including against the direction of any hypothetical convective flow. Finally, we also demonstrate retrograde diffusion of bile salts from intralobular bile ducts into the canalicular network by diffusion.
These results indicate that the movement of bile salts is largely diffusion-dominated and limited, and convective flow is either absent or plays an insignificant role in the process. Diffusion dominated bile flux challenges our current assumptions regarding biliary secretion, the mechanism of choleretic drugs and the pharmacokinetics of substances that are excreted via the biliary route.

Keywords: bile flux mechansim, raster image correlation spectroscopy, intravital imaging, cholestasis

Late Breaking Abstracts

SBMC18-118

A theoretical model of hepatic oxidative stress: Bistability explains the increased susceptibility of fatty livers to ischemia/reperfusion injury

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The shortage of donor organs for liver transplantation calls for the extension of donor organ criteria, so that suboptimal grafts, such as fatty livers, can be used for liver transplantation. However, the dilemma lies in the observation that livers with moderate to high fat accumulation (termed steatosis) show an increased susceptibility to intraoperative ischemia/reperfusion injury. This means that during transplantation the donor organ suffers from a lack of oxygen (hypoxia) followed by fast reoxygenation during reperfusion. Despite intense research, we currently do not fully understand the reason why steatotic livers are prone to ischemia/reperfusion injury.
The aim of our work was to unify current knowledge about relevant physiological processes of the hepatic response to ischemia/reperfusion within a mathematical model. This model links key processes of hepatic lipid metabolism to the formation and detoxification of reactive oxygen species, ultimately leading to the generation of cell-damaging lipid peroxidation products. Our model allows the in silico simulation of hypoxia and reoxygenation events for various degrees of hepatic fat content and predicts the level of hepatic lipid peroxidation as a marker of cell damage caused by oxidative stress. By model analysis, we reveal that the increased susceptibility of steatotic livers can be explained by a feedback loop between processes of hydrogen peroxide detoxification and lipid peroxidation production. This interaction pattern finally causes bistable systems behavior in the level of oxidative stress. Here, the first state represents a low level of oxidative stress and occurs in normal, low fat-loaded livers, whereas for steatotic livers the system is directed to the second state with a high level of oxidative stress. This modeling result promotes our understanding of the increased vulnerability of steatotic livers to ischemia/reperfusion injury during transplantation. Theoretically, our proposed mechanism would support the definition of a cut-off level in the degree of steatosis usable for transplantations: Going over this limit would increase the risk for severe, postoperative liver damage.

Keywords: steatosis, mathematical model, hepatic lipid metabolism, oxidative stress, liver transplantation, ischemia/reperfusion injury

SBMC18-120

Network analysis reveals heterogeneous redox responses in hepatocellular carcinoma patients

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Redox metabolism is often considered a potential target for cancer treatment, but a systematic examination of redox responses in hepatocellular carcinoma (HCC) is missing. Here, we employed systems biology approaches integrating omics data to reveal key roles of genes associated with redox metabolism in HCC. We found that several redox genes, including 25 novel potential prognostic markers, are significantly co-expressed with liver-specific genes and genes associated with immunity and inflammation. HCC tumors display antagonistic behavior in redox responses. The two HCC groups are associated with altered fatty acid, amino acid, drug and hormone metabolism, differentiation, proliferation, and NADPH-independent vs -dependent antioxidant defense. Redox behavior varies with tumor subtype and progression, affecting patient survival. These antagonistic responses are also displayed at the metabolomic and protein level, and were validated in several independent cohorts. Experiments in mice reinforce the observed differential redox behavior, associated with hypoxic features of the two redox gene groups. Our integrative approaches highlighted mechanistic differences among tumors and identified a novel survival signature and potential subgroup-specific therapeutic targets for HCC treatment.

Keywords: Hepatocellular carcinoma, redox metabolism, systems biology, antioxidants, precision medicine, cancer

SBMC18-121

INetMO: an interactive web resource for network visualization of the relationships of multi-omics data

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Recently, there is a big influx of personalized multi-omics data, and this facilitates investigation of the biological relationships among different omics data. Here, we incorporated clinical and multiple omics data, e.g. metabolomics, proteomics and metagenomics data, from recent studies, and built an interactive web resource for network visualization of the relationships of multi-omics data (INetMO). We generated several different networks from each study, which includes general, gender specific and insulin sensitivity networks based on either cross- and delta-correlations. These networks can be employed for assisting
analysis of multi-omics data, suggest potential novel biomarkers and eventually be used in the development of efficient treatment strategies. Moreover, comparative analysis of the networks may allow for the detection of biomarker that could be used in diagnosis in a personalized manner. The networks are presented in interactive website *http://multiomics.inetmodels.com* without any limitation.

Keywords: network, interactive, database, multi, omics, clinical, metabolomics, proteomics, metagenomics

SBMC18-122

A pathology atlas of the human cancer transcriptome

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Cancer is one of the leading causes of death, and there is great interest in understanding the underlying molecular mechanisms involved in the pathogenesis and progression of individual tumors. We used systems-level approaches to analyze the genome-wide transcriptome of the protein-coding genes of 17 major cancer types with respect to clinical outcome. A general pattern merged: Shorter patient survival was associated with up-regulation of genes involved in cell growth and with down-regulation of genes involved in cellular differentiation. Using genome-scale metabolic models, we show that cancer patients have widespread metabolic heterogeneity, highlighting the need for precise and personalized medicine for cancer treatment. All data are presented in an interactive open-access database (*www.proteinatlas.org/pathology*) to allow genome-wide exploration of the impact of individual proteins on clinical outcomes.

SBMC18-126

TMT-based quantitative proteomics reveals the molecular basis of western diet induced liver damage in mice

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Acute on chronic liver failure (ACLF) is characterized by hepatic decompensation resulting in organ/system failure(s) and short-term mortality. To understand the molecular mechanisms contributing to the pathogenesis of ACLF, an animal model of chronic liver disease, based on western diet (WD), was established in the present study. Mice of 4, 6 and 8 weeks of age were fed with the WD and liver tissue as well as blood samples were compiled in 5 different time points (8, 12, 16, 20 and 26 weeks of feeding). Age matching mice were taken as control for each time point. In total, 77 liver tissues and 40 plasma samples were collected and quantitatively investigated at the proteome level by using tandem mass tags (TMT) combined with LC-MS/MS technology to provide a comprehensive understanding of hepatic response to

WD. More than 4000 proteins were identified in liver tissues from which 2631 were successfully quantified. Moreover, 800 proteins were identified after plasma analysis, from which 237 were quantified. All quantified proteins were considered for further data analysis performed by applying the Generalized Additive Models with smooth functions and the Model-based Gene Set Analysis enrichment for active pathway probability calculation. Taken together, our results show that mice at 6 and 8 weeks of age present similar biological processes at the functional level with comparable pattern of regulated proteins, suggesting that these are the best suitable starting points for WD feeding. Additionally, we observed an enrichment of the ribosome pathway in all WD starting points, while an enrichment of amino acid metabolism and extracellular matrix glycoproteins pathways was identified in mice fed with the WD at the age of 6 and 8 weeks.

Keywords: quantitative proteomics, TMT, chronic liver disease, western diet, liver tissue, plasma

SBMC18-128

Systems Approaches to Metabolic Signaling

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The signalling network converging on phosphoinositide-3-kinases (PI3K) and mammalian/mechanistic target of rapamycin (mTOR) controls cellular growth and metabolism and is a central determinant of ageing and age-related diseases. mTOR responds to growth factors, nutrients, cellular energy, and a variety of stresses and controls virtually all anabolic processes including protein and nucleotide biosynthesis as well as lipid metabolism. While the core structure of the PI3K-mTOR network is well established, its wiring and dynamic behaviour differ depending on metabolic conditions as well as cell and tissue context. To systematically explore novel cues and connectivities across the PI3K-mTOR network, we combine detailed and omics wide computational and experimental analyses. Our current work elucidates the network structure in response to amino acids and stresses.

Keywords: mTORC1, stress, computational modeling, metabolism, signaling, cancer

SBMC18-129

Revealing the potential modulation effect of liver-specific lipid metabolism-related genes

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The pathogenesis of nonalcoholic fatty liver disease (NAFLD) and hepatocellular carcinoma (HCC) has been associated with the PKLR, PNPLA3, and PCSK9. Here, we systematically investigated the global effect of inhibition or overexpression of these genes in HepG2 cells with corresponding transcriptome data before and after perturbation and revealed the altered biological functions using integrative network analysis. We observed regulatory and metabolic changes and found that the modulation of the liver-specific genes affected the key pathways involved in the progression of NAFLD and HCC. Here we concluded that potential inhibition of these genes can be used in the development efficient treatment strategy for such complex diseases.

SBMC18-131

Genome-scale metabolic modeling revealed micro-environmental effects on pathogenic bacteria of cystic fibrosis lung

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Cystic fibrosis is the most common autosomal recessive inherited disease among Caucasians. Its inevitable causes of morbidity and mortality are repeated bacterial infections in the lungs caused mainly by Pseudomonas aeruginosa, Staphylococcus aureus, Burkholderia cepacia complex and Haemophilus influenzae. However, the interactions among these bacteria, as well as with their environment, are currently unclear. Systems biology approaches are efficient tools to explore microbial communities. Here, we used genome-scale metabolic models for these bacteria, coupled with synthetic lethality and growth analysis to identify common and distinct growth requirements among these microbes. We observed substantial differences in pathway response to different aerobic growth conditions, alternative pathway susceptibility to extracellular nutrient availability, and different growth requirements among the 4 bacteria. These observations suggest that the microenvironment of the biofilm influence the activities of these microorganisms. Altogether, our observations highlight newly identified important microbial relationships and point towards potential new antimicrobial targets for this disease.

Keywords: cystic fibrosis; host-microbiome interactions; genome-scale metabolic models; systems biology

SBMC18-132

Revealing the molecular mechanism of NAFLD through the use of Genome-scale metabolic models

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Non-alcoholic fatty liver disease (NAFLD) is recognized as liver manifestation of metabolic syndrome, accompanied with excessive fat accumulation in the liver and other vital organs.
Ectopic fat accumulation was previously associated with negative effects at the systemic and local level in the human body. Thus, we aimed to identify and assess the predictive capability of novel potential metabolic biomarkers of ectopic fat depots as well as to observe the metabolic perturbations important for appearance and progression of NAFLD in human subjects. Measured ectopic fat depots were profiled and predicted using a Random Forest algorithm, and by estimating the Area Under the Receiver Operating Characteristic curves, while perturbations in liver metabolism were observed through the use of genome-scale metabolic models. We have identified distinct metabolic signatures of fat depots in the liver (TAG50:1, glutamate, diSM18:0 and CE20:3), pericardium (N-palmitoyl-sphinganine, HGF, diSM18:0, glutamate and TNFSF14), epicardium (sphingomyelin, CE20:3, PC38:3 and TNFSF14), and myocardium (CE20:3, LAPTGF-1, glutamate and glucose). Moreover, glutamate was observed to be important metabolite in NAFLD progression together with the transcriptionally changed genes (ASNS, ALDH18A1, NADSYN1, BCAT1, GOT1, GLS, PSAT1, FTCD and AADAT). Our analyses highlight non-invasive biomarkers that accurately predict ectopic fat depots, and reflect their distinct metabolic signatures in subjects with NAFLD. In addition, glutamate was recognized as a prominent metabolite undergoing significant transcriptional changes in the appearance and the progression of NAFLD

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