

HeCaToS Newsletter

Hepatic and Cardiac Toxicity Systems modelling (HeCaToS)

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HeCaToS consortium photo at the 3rd annual meeting in Valencia, Spain

The 3rd HeCaToS Annual Consortium Meeting was successfully held at the Instituto de Investigación Sanitaria La Fe, Valencia, Spain from 2nd to 4th November, 2016. The three-day meeting was fully packed with high quality presentations all around from our consortium partners. The meeting on the first day included parallel work package meetings in the morning, and a focus session in the afternoon on developing HeCaToS's proof-of-concept cardiotoxicity prediction models.

The key emphasis of the afternoon session was to pull together resources from the modelling expertise and omics analyses, strengths across the HeCaToS work packages, and the focus session helped yield many productive discussions.

Meeting on the second day featured detailed updates on results from all the work packages, and the diversity of the work carried by our project partners ranging from Computational chemistry, Molecular pathway modelling and physiological modelling, Vertical model integration, In-vitro assays, Clinical sample resources, Omics and Functional analyses, Data warehouse and quality control, and Integrated statistical and predictive comparisons were all represented. The final day of the programme consisted of a series of omics analyses and database training workshops given by Dr. Ralf Herwig (Max Planck Institute for Molecular Genetics, Germany), Jasmine Minguet (EBML-EBI, UK), and Dr. Hans Gmuender (Genedata, Switzerland). The meeting also featured a plenary talk by Dr. Russell Scot Thomas from the National Centre for Computational Toxicology at the US-EPA, who has given us an update on the recent EPA efforts in omics analyses, in-vitro testing and predictive modelling. Also, our annual meeting benefited from a vibrant poster session featuring excellent work from our partner members. The poster 1st prize was awarded to Sandra Tejedor (Instituto de Investigación Sanitaria La Fe, Spain) and the 2nd prize went jointly to Dr. Alex Lewalle (Kings College London, UK) and Olivia Clayton (Roche, Switzerland). We also want to thank Prof. Jose Castell for hosting the annual meeting and for organising a city bus tour and the consortium dinner at Restaurant Submarino Oceanográfico inside Valencia's Aquarium for the meeting participants.

Genomics and toxicity prediction with Dr. Ralf Herwig

By Dr. Chung-Ho Lau (Imperial College London, UK)

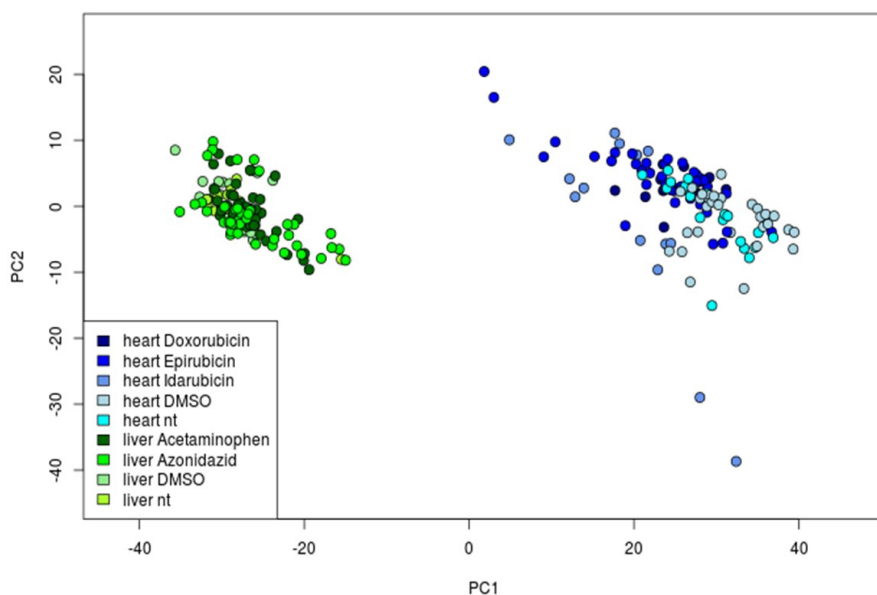
Bioinformatics and genome analysis play key roles in providing us deep molecular insights into drug toxicity at various levels of cellular information. Dr. Ralf Herwig (Max-Planck-Institute for Molecular Genetics, Germany) has been working in genome analysis since the days of the Human Genome Project and develops statistical tools for sequence and network analysis as a pre-requisite for systems biology. I interviewed Dr. Herwig to learn more about his work:

Why genome analysis in toxicology?

High-throughput sequencing or, as many people name it, next generation sequencing has revolutionized our view on molecular biology. Nowadays, it has become possible and affordable to sequence a complete genome in days rather than in years as it was the case before. This has given rise to the massive study of the so-called 'omics', the genome, methylome, and transcriptome of a cellular system of interest, what allows deep characterization and ultimately a better understanding of the system. Analyzing drug-induced changes of these landscapes allows prediction of why it may be toxic in a particular cellular setting and, thus helps defining its mode of action.

What is the impact of bioinformatics for genome analysis?

High-throughput sequencing is not achievable without bioinformatics. A typical sequencing experiment generates hundreds of millions to billions of short sequences which have to be processed, aligned and analyzed in order to find mutations, aberrant gene expression or methylation sites. Without the use of robust bioinformatics methods this is simply not possible. Furthermore, these bits of information have to be set into biological context. Our approach to discover this context is to utilize *a priori* information based on molecular networks. We use known signaling and interaction networks and map the genomic read-outs onto these networks. Mathematical graph theory then gives us efficient algorithms for inferring these networks in order to identify potential drug targets or drug adverse outcome pathways.



Left: Multivariate analysis of promoter methylation shows that hepatic (green) and cardiac (blue) microtissues are associated with distinct phenotypes.

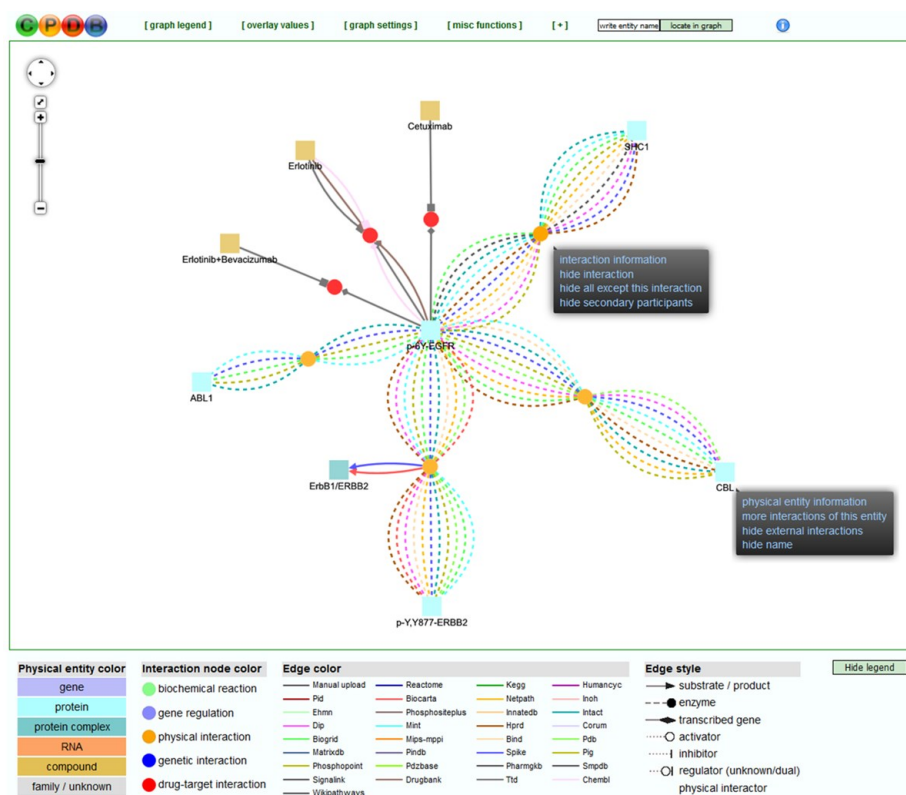


Dr. Ralf Herwig (Max-Planck-Institute for Molecular Genetics)

Genomics and toxicity prediction with Dr. Ralf Herwig

What are you currently working on?

We are developing methods for genome-wide methylation analysis. Very recently, we have developed a statistical approach to predict the level of methylation of CpG sites from sequencing counts which is highly accurate and outperforms previously published methods (Lienhard et al., 2016). In HeCaToS we use this method to identify drug-induced differentially methylated promoters in microtissues and to predict the effect of these methylation changes on gene and protein expression. Additionally, we have built and host the ConsensusPathDB interaction resource which integrates >30 public network and pathway repositories and is presumably one of the largest interaction resources worldwide (Herwig et al., 2016). In HeCaToS we use this resource for building interaction networks and pathway gene/protein/metabolite sets as the basis for interpreting the various omics outcomes. The research is carried out by two PhD students in my group, Matthias Lienhard and Gal Barel.



Visualising the EGFR interaction network through ConsensusPathDB (<http://consensuspathdb.org>)

References

- Lienhard M, Grasse S, Rolff J, Frese S, Schirmer U, Becker M, Böro S, Timmermann B, Chavez L, Sülthmann H, Leschber G, Fichtner I, Schweiger MR, Herwig R. QSEA-modelling of genome-wide DNA methylation from sequencing enrichment experiments. *Nucleic Acids Res*. 2016 Dec 1. pii: gkw1193.
- Herwig R, Hardt C, Lienhard M, Kamburov A. Analyzing and interpreting genome data at the network level with ConsensusPathDB. *Nat Protoc*. 2016 Oct;11(10):1889-907.

Proteome profiling for the comprehensive characterization of cell and tissue states

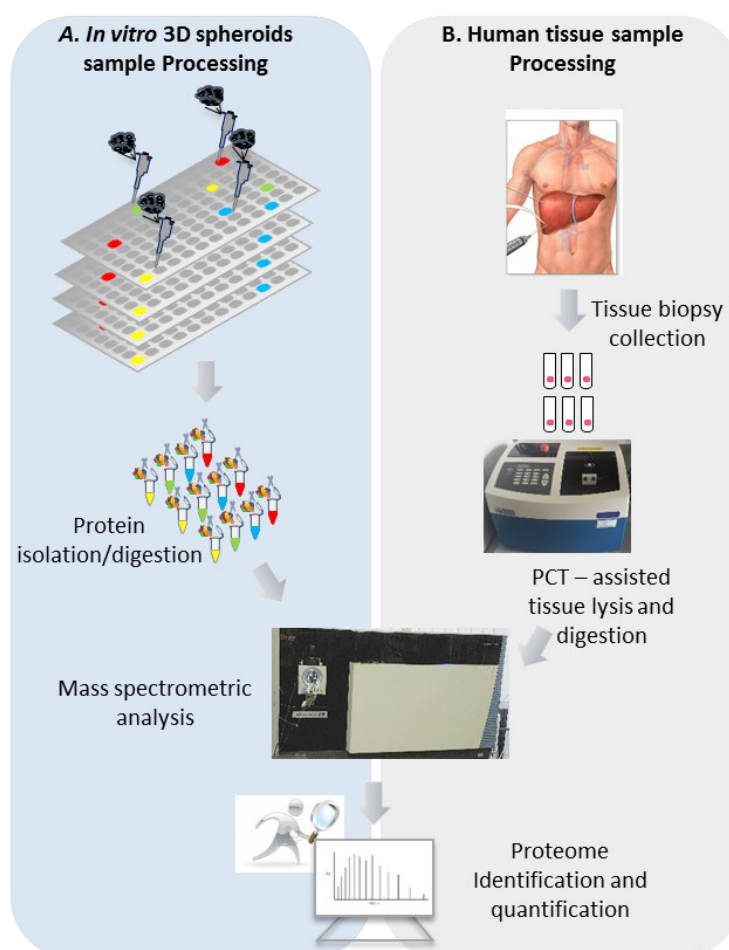
By Dr. Nathalie Selevsek and Prof. Dr. Ralph Schlapbach (ETH Zurich, Switzerland)

By applying proteomics – based workflows, we aim to elucidate the entire content of proteins in cells, organs or organisms, including protein abundances, post-translational modifications (PTMs) and protein-protein interactions. Challenges arise from the sample complexity and the protein dynamic range that differs between organs or cell types, changes over time and in response to physiological conditions.

The fast development of new instrumentation and software tools have made mass spectrometry (MS)-based proteomics a prerequisite for the investigation of molecular and cellular processes. Exploiting the possibility to take protein fragments, i.e. peptides, and ionize and further fragment those in the mass spectrometer, allows to generate multidimensional MS data. Thanks to the tremendous improvement in MS sensitivity, resolution and accuracy of the data, we are able to determine protein identity, quantify their abundance (ie. relative as well as absolute), and decipher their multiple proteoforms in single experiments within a few hours of measuring time.

For HeCaToS, we implemented a highly efficient sample preparation protocol that allows us to work with minute amounts of sample material as provided by the 3D spheroid liver and heart microtissues and patient tissue biopsies. Combining a label-free quantitative proteomics strategy with powerful bioinformatics analysis, thousands of proteins can be identified and quantified across the hepatocardiotoxicant challenged biological samples, revealing several pathways connected to organ failure.

As a continuation of the HeCaToS efforts, we are currently expanding the PCT (pressure cycling technology) assisted tissue lysis and digestion and subsequent analysis efforts towards even more comprehensive mapping of the proteome, leading to a more complete coverage of metabolic and protein interaction maps and networks known. We also optimize the analysis of the alternative molecular subfractions isolated from the tissue samples for the characterization of metabolites (metabolomics) and gene expression (transcriptomics) by again MS and Next Generation Sequencing (NGS) technologies, respectively.



Proteomics profiling workflow

Who's who?



Prof. Dr. Stephane Heymans is Professor of Cardiomyopathies and Head of the Centre for Heart Failure Research, Department of Cardiology, Maastricht University. In 2003, He joined the Cardiology Department University Hospital of Maastricht (The Netherlands) as an academic medical specialist and in 2006 he took over the Multidisciplinary Cardiomyopathy Program (Genetics, Immunology, Microbiology and Pathology departments). His research interests focus on the molecular mechanisms of heart failure, looking at the interplay between inflammatory cells, fibroblasts and cardiomyocytes. Particular focus is on the role of non-structural matrix proteins, matrix turnover and non-coding RNAs.



Dr. Alex Lewalle is based in the Cardiac Electromechanics Research Group at King's College London. After completing his PhD in solid-state physics, he redirected his research interests to the physics/biology interface, focusing on the general theme of force generation and mechanical behaviour in biological systems. His work has involved both experimental measurement and theoretical modelling on multiple length scales, aiming to understand the mechanisms underlying physiological behaviour by bridging experimental and theoretical approaches. Within HeCaToS, he seeks to integrate clinical and experimental measurements into computational models to investigate the impact of cardiotoxicity on cardiac physiology.



Jasmine Minguet obtained a Licence in Experimental Biomedical Sciences and a Master in bioinformatics, from the Université catholique de Louvain of Brussels, Belgium. She previously worked six years for the Belgian army in a molecular biology laboratory, developing pathogen detection tests. Jasmine joined HeCaToS project in May 2015. She works now at the European Bioinformatics Institute in Dr. Ugis Sarkans's team, as a data manager and is responsible for the creation of metadata templates, metadata harmonization, management of heterogeneous datasets, and feeding data into the BioStudies database.

Recent Project Publications

A high number of articles have been published by our project partners since the previous issue of the HeCaTos Newsletter in October last year, demonstrating the important findings and impacts of our project studies. Many congratulations to all the authors involved.

Funding from the HeCaToS project has been acknowledged in the following publications:

de Oliveira, B. L.; Niederer, S., A Biophysical Systems Approach to Identifying the Pathways of Acute and Chronic Doxorubicin Mitochondrial Cardiotoxicity. *Plos Computational Biology* 2016, 12 (11).

Cordes, H.; Thiel, C.; Aschmann, H. E.; Baier, V.; Blank, L. M.; Kuepfer, L., A Physiologically Based Pharmacokinetic Model of Isoniazid and Its Application in Individualizing Tuberculosis Chemotherapy. *Antimicrobial Agents and Chemotherapy* 2016, 60 (10), 6134-6145.

Herwig R, Hardt C, Lienhard M, Kamburov A. Analyzing and interpreting genome data at the network level with ConsensusPathDB. *Nat Protoc.* 2016;11(10):1889-907.

Thiel, C.; Cordes, H.; Fabbri, L.; Aschmann, H. E.; Baier, V.; Smit, I.; Atkinson, F.; Blank, L. M.; Kuepfer, L., A Comparative Analysis of Drug-Induced Hepatotoxicity in Clinically Relevant Situations. *Plos Computational Biology* 2017, 13 (2).

Thiel, C., Cordes, H., Baier, V., Blank, L. and Kuepfer, L. Multiscale modeling reveals inhibitory and stimulatory effects of caffeine on acetaminophen-induced toxicity in humans. *CPT Pharmacometrics Syst. Pharmacol.*, (2017), 6: 136–146

Tyzack, J. D.; Hunt, P. A.; Segall, M. D., Predicting Regioselectivity and Lability of Cytochrome P450 Metabolism Using Quantum Mechanical Simulations. *Journal of Chemical Information and Modeling* 2016, 56 (11), 2180-2193.

Heggermont, W. A.; Papageorgiou, A. P.; Heymans, S.; van Bilsen, M., Metabolic support for the heart: complementary therapy for heart failure? *European Journal of Heart Failure* 2016, 18 (12), 1420-1429.

Coloma, C. S.; Sepulveda, P.; Hernandez, A.; Tejedor, S.; Palomar, L.; Ruiz, A.; Miro, V.; De la Cueva, H.; Ontoria-Oviedo, I.; Salvador, A.; Castel, V.; Santaballa, A., Anthracycline mediated cardiotoxicity: Detection of miRNA based early biomarkers for the prediction of myocardial injury. Hecatos study. *Annals of Oncology* 2016, 27.

Siskos, A. P.; Jain, P.; Romisch-Margl, W.; Bennet, M.; Achaintre, D.; Asad, Y.; Marney, L.; Richardson, L.; Koulman, A.; Griffin, J. L.; Raynaud, F.; Scalbert, A.; Adamski, J.; Prehn, C.; Keun, H. C., Interlaboratory Reproducibility of a Targeted Metabolomics Platform for Analysis of Human Serum and Plasma. *Analytical Chemistry* 2017, 89 (1), 656-665.