International Workshop
ENVIRONMENTAL OMICS INTEGRATION & MODELING

CosmoCaixa, BARCELONA October 18-20, 2017

In collaboration with:

Sponsored by:

WELCOME

PRESENTATION

The aim of this workshop is to review the state-of-the-art and broaden the knowledge on high-throughput analytical methods, data integration and modeling in Environmental Omics and Toxicology.

The main workshop topics will be the following:

- **Transcriptomic and Genomic studies**
- **Metabolomic and Lipidomic studies**
- **Development of Chemometrics and Analytical Tools**
- **Data Integration and Modeling**

We are pleased to invite you to participate in this workshop that will promote knowledge exchange and creation of new relationships and future collaborations.

ORGANIZING COMMITTEE:

This workshop is organized by researchers from the Institute of Environmental Assessment and Water Research (IDAEA-CSIC) under the scope of the ERC Advanced Grant (320737-CHEMAGEB) that aims to develop new chemometric and high-throughput analytical methods for Environmental Omics and Toxicology.

This act is also organized as part of the commemorative acts of the Research and Development Center (CID) 50th anniversary.

Carmen Bedia
Bruno Campos
Cristian Gomez-Canela
Igor Marín de Mas
Laia Navarro Martin
Romà Tauler

Website: wenvomics2017.info

PROGRAM

Wednesday, October 18th, 2017

13.00 WORKSHOP REGISTRATION

15.00-15.30 WORKSHOP PRESENTATION AND OPENING

(**Romà Tauler**, IP of the ERC Advanced Grant CHEMAGEB project, and **Joan Grimalt**, IDAEA and CID director).

SESSION 1: GENOMICS AND TRANSCRIPTOMICS

Chairs: **Benjamín Piña**, **Carlos Barata** and **Laia Navarro**. IDAEA-CSIC.

PLENARY LECTURES

15.30-16.00

"Statistical machine learning: routes to discovery in toxicology and ecotoxicology from multi-omics data".

James B. Brown. University of Birmingham (Birmingham, UK) and University of California, Berkeley (California, USA).

Research Interests: Genomics, Ecotoxicology, Statistical Machine Learning, Molecular Ecosystems Biology.

16.00-16.30

"Environmental Genomics in a Variable World: Defining the issues".

Christopher J. Martyniuk. Center for Environmental and Human Toxicology & Department of Physiological Sciences (Florida, USA).

Research Interests: Toxicology, Neuroendocrinology, Neurogenic Hypertension, Transcriptomics, Proteomics, Endocrine Disruption.

16.30-17.00 COFFEE BREAK

Wednesday, October 18th, 2017

SHORT COMMUNICATIONS

17.00-17.20

"Methylation sensitive high resolution melting as a high throughput technique to detect DNA methylation alterations after exposures to endocrine disruptors"

Laia Navarro-Martín. IDAEA-CSIC, Barcelona

17.20-17.40

"Development of microbial indicators of anaerobic digestion inhibition with omics"

Olivier Chapleur. IRSTEA, Antony, France

17.40-18.00

"Transcriptomic responses to temperature shifts in a mediterranean keystone sea urchin"

Rocío Pérez-Portela. CEAB-CSIC, Blanes, Spain

18.00-18.10- VENDOR SEMINAR

"Comprehensive targeted and non-targeted analysis of various indoor dust samples using LC-HRMS with Ion Mobility"

Pablo de la Iglesia (Applications Specialist - Waters)

FLASH-POSTER PRESENTATIONS

18.10-18.15 P01

"The human early life exposome (HELIX) project : Molecular Mechanisms. "

Marta Vives-Usano. Centre for Genomic Regulation (CRG), Universitat Pompeu Fabra (UPF), Barcelona, Spain

18.15-18.20 P02

"Untargeted liquid chromatography coupled with high resolution mass spectrometry analysis of rice lipidome under hydric and heat stress"

Meritxell Navarro-Reig. Institute of Environmental Assessment and Water Research, CSIC, Barcelona, Spain

Wednesday, October 18th, 2017

18.20-18.25 P03

"Assessment of endocrine disruptors effects on zebrafish embryos by untargeted metabolomics"

Elena Ortiz-Villanueva. Institute of Environmental Assessment and Water Research, CSIC, Barcelona, Spain

18.25-18.30 P04

"Exposure to chlorpyrifos causes morphometric, biochemical and lipidomic alterations in green beans (Phaseolus vulgaris)"

Carmen Bedia. Institute of Environmental Assessment and Water Research, CSIC, Barcelona, Spain

18.30-18.35 P05

"Untargeted lipidomic study of chronic UV radiation effects in melanocytes"

Núria Dalmau. Institute of Environmental Assessment and Water Research, CSIC, Barcelona, Spain

18.35-18.40 P06

"ROIMCR: a powerful data analysis strategy for LC-MS metabolomic data sets"

Eva Gorrochategui. Institute of Environmental Assessment and Water Research, CSIC, Barcelona, Spain

18.40-18.45 P07

"Untargeted metabolomic processing of blood samples from preterm infants stored with RNA stabilization reagent"

José David Piñeiro-Ramos. Instituto de Investigación Sanitaria La Fe (IIS La Fe), Valencia, Spain

18.45-18.50 P08

"Analysis of neurobehavioural data by chemometric methods in ecotoxicological studies"

Cristian Gómez-Canela. Institute of Environmental Assessment and Water Research, CSIC, Barcelona, Spain

Wednesday, October 18th, 2017

18.50-18.55 P09

”The hide-and-seek challenge of 1H-13C HSQC NMR Metabolomics ”

Francesc Puig-Castellví. Institute of Environmental Assessment and Water Research, CSIC, Barcelona, Spain

18.55-19.00 P10

”Muscle lipids of fish may be affected by environmental pollution ? ”

Maria Blanco. Institute of Environmental Assessment and Water Research, CSIC, Barcelona, Spain

19.15 SOCIAL ACTIVITY : PLANETARIUM SESSION

Thursday, October 19th, 2017

SESSION 2: METABOLOMICS AND LIPIDOMICS

Chairs: **Cristian Gómez**, **Sílvia Lacorte** and **Carmen Bedia**. IDAEA-CSIC.

PLENARY LECTURES

9.00-9.30

"Environmental Metabolomics: New Tools and Techniques to Measure the Exposome".

David Wishart. Departments of Computing Science and Biological Sciences, University of Alberta (Edmonton, Canada).

Research Interests: Bioinformatics, Metabolomics, Structural Biology, Nanobiology, Synthetic Biology and Prion Biology.

9.30-10.00

"Metabolomics and Systems Biology in studies on Endocrine Disruptors".

Daniel Zalko. Institut national de la recherche agronomique, INRA (Toulouse, France).

Research Interests: Metabolism of Xenobiotics, Metabolic Networks, Metabolism, Genotoxicity of Xenobiotics.

10.00-10.30

"Efficacy of spectrochemical biomarkers to identify sublethal stress in environmental settings".

Francis L Martin. Pharmacy and Biomedical Sciences, University of Central Lancashire (Preston, UK).

Research Interests: Genetic toxicology, bio-imaging and biospectroscopy.

10.30-11.00 COFFEE BREAK

Thursday, October 19th, 2017

SHORT COMMUNICATIONS

11.00-11.20

"Metabolomics reveals metabolic disorders in mice exposed to thirdhand tobacco smoke"

Noelia Ramírez. University Rovira i Virgili, Tarragona, Spain

11.20-11.40

"Re-thinking ecotoxicology paradigms in the omics era"

Steven D. Melvin. Griffith University, Southport, Australia

11.40-12.00

"Environmental effects of cyanobacterial blooms on fish: a metabolomic approach from experimental fish to natural population"

Benjamin Marie. Muséum National d'Histoire Naturelle, Paris, France

12.00-12.20

"Hybrid target/non-target profiling of biological samples for human metabolomics & exposomics studies"

James J. Harynuk. University of Alberta, Edmonton / Canada

12.20-12.40

"Identifying markers of lung cancer and cardiovascular disease with adductomics"

Sonia Dagnino. Imperial College London, London, UK

Thursday, October 19th, 2017

FLASH-POSTER PRESENTATIONS

12.40-12.45 P11

" GPCA for improved multivariate analysis interpretation in lipidomics"

Sara Tortorella. Molecular Horizon, Perugia, Italy

12.45-12.50 P12

"Mercury effects in salt marsh plants: alterations in the metabolome of Halimione portulacoides from a highly contaminated system"

Célia Fernandes. Department of Biology, University of Aveiro, Aveiro, Portugal

12.50-12.55 P13

"Metabolic flux analysis of glucose-lactate concomitant consumption in cho cell cultures "

Iván Martínez-Monge. Universitat Autònoma de Barcelona, Bellaterra, Spain.

12.55-13.00 P14

"Bacterial volatiles as plant growth promoters-detection by the combined application of HS-SPME, GC-MS and MCR-ALS analysis "

Paulo Cardoso. Department of Biology and CESAM, University of Aveiro, Portugal

13.00-13.05 P15

"Identifying biomarkers by integrating multiple-omics datasets to improve anaerobic digestion"

Olivier Chapleur. Hydrosystems and Bioprocesses Research Unit, IRSTEA, Antony, France

13.05-13.10 P16

"Combined use of chemometrics and hyperspectral images for enviromental –omics studies applied to rice plants"

Silvia Concolino. Department of Chemical Engineering and Analytical Chemistry, University of Barcelona, Barcelona, Spain.

LUNCH & POSTER SESSION

Thursday, October 19th, 2017

SESSION 3: CHEMOMETRICS

Chairs: **Anna de Juan** (Universitat de Barcelona) and **Joaquim Jaumot** (IDAEA-CSIC).

PLENARY LECTURES

15.00-15.30

"Common and Distinct Components in Data Fusion".

Age Smilde. Biosystems Data Analysis, Swammerdam Institute for Life Sciences, University of Amsterdam (Netherlands).

Research Interests: Multiset data analysis and implementation in fields of biology.

15.30-16.00

"Approaches for the analysis of designed metabolomic data".

Federico Marini. Dept. of Chemistry, University of Rome "La Sapienza" (Rome, Italy).

Research Interests: Analytical Chemistry, Chemometrics.

16.00-16.30

"Multivariate analysis and integration of multi-OMICS data".

Johan Trygg. Kemiska institutionen Umeå universitet, (Umeå, SE).

Research Interests: Chemometrics in Metabolomics, Systems biology and Process Analytical Technologies.

16.30-17.00 COFFEE BREAK

Thursday, October 19th, 2017

SHORT COMMUNICATIONS

17.00-17.20

"A new omics for the environment: algal single-cell fingerprinting with flow cytometry"

Jeroen Jansen. Institute for Molecules and Materials (IMM), Nijmegen, The Netherlands

17.20-17.40

"Regularized MANOVA for analysis of untargeted multi-factor metabolomics data"

Jasper Engel. Institute for Molecules and Materials (IMM), Nijmegen, The Netherlands

17.40-18.00

"Urinary metabolome changes in preterm newborns fed with breast milk or donor breast milk"

Julia Kuligowski. Instituto de Investigación Sanitaria La Fe (IIS La Fe), Valencia, Spain

18.00-18.20

"Hyperspectral imaging and chemometrics for environmental –omics"

Anna de Juan. Universitat de Barcelona, Barcelona, Spain.

Thursday, October 19th, 2017

FLASH-POSTER PRESENTATIONS

18.20-18.25 P17

"Assessment of the effect of time exposure of chlorpyrifos-oxon on different phenotypes of zebrafish embryos by hyperspectral imaging and chemometric methods"

Víctor Olmos. Department of Chemical Engineering and Analytical Chemistry, University of Barcelona, Barcelona, Spain.

18.25-18.30 P18

"Modelling the duration of hypoxia: a plasma metabolite score. "

Daniel Cuesta García. Health Research Institute Hospital La Fe, Valencia, Spain

18.30-18.35 P19

"Evaluation of batch effect elimination using quality control replicates in LC-MS metabolite profiling"

Guillermo Quintás. Leitat Technological Center, Valencia, Spain

18.35-18.40 P20

"Outfitting the factory of the future with on-line analysis"

André van den Doel. Institute of Molecules and Materials, Radboud University, The Netherlands

18.40-18.45 P21

"Bisphenol A (BPA) as a disrupter of lipid metabolism in zebrafish embryos "

Rubén Martínez. Dept. of Environmental Chemistry, IDAEA-CSIC, Barcelona, Spain

18.45-18.50 P22

"Altered lipid metabolism in Daphnia magna exposed to ecdysteroids, juvenoids and Bisphenol A "

Inmaculada Fuertes. Dept. of Environmental Chemistry, IDAEA-CSIC, Barcelona, Spain

20:30 GALA DINNER

Restaurante La Balsa

Friday, October 20th, 2017

SESSION 4: DATA INTEGRATION AND MODELING

Chairs: **Romà Tauler** (IDAEA-CSIC) and **Igor Marín de Mas** (Danmarks Tekniske Universitet).

PLENARY LECTURES

9.00-9.30

"Multiomics data integration to unravel local gene regulatory networks".

Ana Conesa. Intituto Principe Felipe (Valencia, Spain).

Research Interests: Integration of multi-omics data, Genomics of gene expression.

9.30-10.00

"A systems medicine approach to target metabolic reprogramming associated to metastasis".

Marta Cascante. Department of Biochemistry and Molecular Biology, Facultad de Biología, Universitat de Barcelona (Barcelona, Spain).

Research Interests: Integrative Systems Biology, Metabolomics and Cancer.

10.00-10.30

"Kinetic modelling within our GRASP: new strategies for fitting accurate network kinetic models".

Lars Keld Nielsen. Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark (Lyngby, Denmark).

Research Interests: Systems and Synthetic Biology.

10.30-11.00 COFFEE BREAK

11.00-11.30

"Enhancing integrative omics analysis with biological knowledge".

Alex Sánchez-Pla. Genetics Microbiology & Statistics Department, University of Barcelona and Statistics and Bioinformatics Unit, Vall d'Hebron Institut de Recerca.

Research Interests: Integrative Omics Analysis and Biomarker discovery.

Friday, October 20th, 2017

SHORT COMMUNICATIONS

11.30-11.40- Vendor Seminar

"New Solutions For Next-Gen Lipidomics".

Isidro Griful. Sciex Instrument Sales Specialist

11.40-12.00

"Study of the effects of chronic exposure to aldrin in prostate cancer via multi-level approaches".

Igor Marín de Mas. Danmarks Tekniske Universitet. Lyngby, Denmark.

12.00-12.20

"Rexposome: a bioinformatic tool for characterizing multiple environmental factors and its association with different omics biomarkers and disease"

Carles Hernandez-Ferrer. CREAL, UPF, CIBERESP, Barcelona, Spain

12.20-12.40

"Multidataset: an R package for encapsulating multiple data sets with application to omic data integration"

Juan R. González. CREAL, UPF, CIBERESP, Barcelona, Spain

12.40-13.00 CLOSING REMARKS

INVITED SPEAKERS

JAMES B. BROWN

University of Birmingham (Birmingham, UK) and University of California, Berkeley (California, USA).

Ben Brown's lab works to link genome dynamics to ecosystem functions – to understand how the information encoded in genomes gives rise to complex, and profoundly responsive systems. Enormous progress has been made linking genes to cellular, tissue, organ, and organismal systems. The grand challenge before us is to understand the impact of genes beyond the organism; to understand gene function in ecological contexts. This emerging field is known as "molecular ecosystems biology", and many advances are needed to enable its emergence as a foundational science. The Brown lab works to develop analytical capabilities on the following fronts:
Deep model organisms: species where we work systematically toward a comprehensive understanding of gene regulation and molecular bionomics, including Drosophila, Daphnia, zebrafish, mouse, human-derived cells, and several microbes. **Model ecologies:** self-sustaining and recapturable systems with defined trajectories can be used to interrogate complex communities with the same felicity we enjoy in the development of model organisms. Examples include the Drosophila gut microbiome (given genotype and culture conditions), and the EcoFAB initiative (http://eco-fab.org/what-is-ecofab/). **Molecular exposure biology:** perturbations of metabolic pathways even when those pathways are spread across consortia involving multiple species or populations. **Phylogenomic reconstructions:** provide translations of results collected in model systems to real-world ecologies. **Nondestructive measurements:** time-courses enable the study of system dynamics, e.g. hyperspectral imaging and minimally invasive sequencing. **Informative Learning Machines:** introspective algorithms used to obtain insight from multi-modal datasets. The Brown Lab is developing this new area of machine learning to integrate pan-omics datasets.

Statistical machine learning: routes to discovery in toxicology and ecotoxicology from multi-omics data

The activities of the gut microbiome under basal conditions have been extensively studied in several species, and include the production of vitamins, nutrients, and neurotransmitters, the modulation of the host immune system, as well as the generation of harmful chemical compounds in a diet-dependent fashion. Recently, it has been shown that the gut microbiome plays a major role in the metabolism of some drugs. Therefore, commensal microbial communities may also mediate the physiological effects of common, real-world chemical exposures. We focused on the widely used herbicides atrazine and paraquat, which both produce adverse health outcomes in humans and keystone pollinators in field exposure scenarios. We applied integrated omics and phenotypic screening to assess the role of the gut microbiome in modulating host resilience to collateral dietary pesticide exposure in Drosophila melanogaster and Mus musculus. Despite the vast evolutionary distance between the two species, we find related transcriptional and metabolic responses to these compounds, which are sex-specific and depend strongly on the presence of the commensal microbiome. In fly, we identify a single bacteria sufficient to convey resistance to atrazine, and isolate the enzymatic pathway responsible for the trait. This work points toward the derivation of biotic strategies to improve host resilience to environmental chemical exposures, and illustrates the power of integrative omics to identify pathways responsible for adverse health outcomes – even when those pathways span multiple trophic levels.

CHRIS J. MARTYNIUK

Center for Environmental and Human Toxicology & Department of Physiological Sciences (Florida, USA).

Dr. Chris J. Martyniuk is an Associate Professor at the University of Florida in the College of Veterinary Medicine. He is a molecular toxicologist and comparative endocrinologist focused on defining adverse outcome pathways for both endocrine and non-endocrine (e.g. mitochondrial bioenergetics) pathways in aquatic organisms. To achieve this, his laboratory uses transcriptomics, proteomics, and network analysis to associate molecular responses to higher levels of biological organization. His research group has worked with a variety of fish species including zebrafish, fathead minnow, largemouth bass, rainbow darter, and shortnose sturgeon, and his research encompasses both laboratory and field research. He is active internationally and is currently a Canadian Rivers Institute Science Director and a former Tier II Canada Research Chair in Molecular Ecology. He is also a member of the Omics Steering Committee for the Society of Environmental Toxicology and Chemistry, is a Councillor for the North American Society for Comparative Endocrinology, and is the Editor-in-Chief of the international publication Comparative Biochemistry and Physiology - Part D: Genomics and Proteomics. He was the inaugural recipient of The Gorbman - Bern New Independent Investigator Award given by the North American Society for Comparative Endocrinology. Dr. Martyniuk has authored or co-authored 120 publications in neurotoxicology, reproductive biology, endocrine disruptors, aquatic toxicology, and bioinformatics.

Environmental Genomics in a Variable World: Defining the issues

Environmental science has benefited a great deal from omics-based technologies. High-throughput toxicology has defined adverse outcome pathways, prioritized chemicals of concern, and identified novel actions for environmental chemicals. While many of these approaches are conducted under rigorous laboratory conditions, a significant challenge has been the interpretation of omics data in “real-world” exposure scenarios. This limits the use of omics data in environmental monitoring programs. Our objective has been to address fundamental questions about experimental design and the robustness of data collected for environmental genomics. Using the Grand River in Canada as an experimental site, our studies have addressed the likelihood that molecular responses return to baseline conditions following wastewater treatment plant upgrades. We also examined how reference site selection in an urban environment influences interpretation of omics data. Other questions surround the optimal selection of monitoring species and tissues for omics endpoints. Based upon a meta-analysis, we determined that transcriptomes of single-spawning fish showed lower variability compared to multiple-spawning species, suggesting that single spawning species may be more useful for monitoring programs. Lastly, to address technical variability in transcriptomics, we and others conducted an inter-genomics study to assess the transcriptome repeatability in different laboratories. Without employing rigorous standardization of sample processing and bioinformatics, we found that two laboratories are ~50-60% likely to identify the same differentially expressed transcripts. However, most important was that all six laboratories identified a predefined set of responsive genes known a priori to be affected by the chemical (i.e., 17alpha-ethyinylestradiol), supporting the idea that monitoring a subset of molecular biomarkers in the field may be useful. Spatial and temporal variability in ecosystem structure can influence molecular responses to stressors and it is important to recognize how these variables, as well as the inherent biological variability in the “omes”, can influence our interpretation of Big Data.

DAVID WISHART

Departments of Biological Sciences and Computing Science, University of Alberta
Director, The Metabolomics Innovation Centre (TMIC)

Dr. David Wishart (PhD Yale, 1991) is a Professor in the Departments of Biological Sciences and Computing Science at the University of Alberta. He also holds adjunct appointments with the Faculty of Pharmaceutical Sciences and with the Department of Pathology and Laboratory Medicine. He has been with the University of Alberta since 1995. For the past 10 years, he has led the "Human Metabolome Project" (HMP), a multi-university, multi-investigator project that is cataloguing all of the known metabolites in human tissues and biofluids. Using advanced methods in NMR spectroscopy, mass spectrometry, multi-dimensional chromatography and machine learning Dr. Wishart and his colleagues have identified or found evidence for more than 70,000 metabolites in the human body. This information has been archived on a freely accessible web-resource called the Human Metabolome Database (HMDB). He has published more than 330 research papers in many different areas including metabolomics, exposomics, bioinformatics, NMR, mass spectrometry, structural biology, and precision medicine. He has also founded successful 6 biotech companies, each of which grew out of his lab's research findings. Dr. Wishart currently directs The Metabolomics Innovation Centre (TMIC), Canada's national metabolomics laboratory. He has been recognized by Thomson-Reuters as one of the worlds most highly cited scientists for each of the last 3 years.

New Tools and Techniques to Measure the Exposome

Over the last 100 years more than 100,000 different chemicals have been isolated or synthesized and incorporated into commercial products such as plasticizers, dyes, chemical solvents, pharmaceuticals, household products, cosmetics, paints, fertilizers, foods and food additives. The chemicals in these products can be readily absorbed by humans, and/or released into the environment, either in their natural or degraded form. These compounds are part of what is now called the chemical "exposome". The chemical exposome refers to the sum total of all chemicals (natural or synthetic) that an individual is exposed to throughout their lifetime. Identifying these chemicals and their biotransformation products is proving to be particularly challenging. The emerging field of environmental metabolomics potentially offers an appealing route to identify these compounds. However, environmental metabolomics is not without some problems. For many environmental samples as well as for many biofluids, less than 5% of the mass spectral (MS) features can be identified. The other 95% of MS features that cannot be identified are often called the "dark matter" of the exposome. In this presentation I will describe a number of databases as software tools that we are developing to help characterize this "dark matter". In particular, I will present: 1) The Human Metabolome Database, the main data resource on chemical compounds (both natural and synthetic) found in humans; 2) ContaminantDB, a comprehensive electronic database of nearly 100,000 known chemical contaminants; 3) BioTransformer, a software tool for predicting metabolic biotransformation products arising from human metabolism and environmental microbial degradation; and 4) CFMID+, a software tool designed to accurately predict mass spectra for rapid compound identification. I will also go through several case studies and provide some specific examples to illustrate how these tools can be used to help address the challenges in MS-based environmental metabolomic and exposomic studies and to assist with more conventional toxicological studies, environmental monitoring and chemical risk assessment.

DANIEL ZALKO

Institut national de la recherche agronomique, INRA (Toulouse, France)

Research Director at the French National Institute for Research in Agronomy (INRA). Toulouse, France). He was trained as a veterinarian and later graduated with a PhD in Toxicology. He heads the "Metabolism of Xenobiotics" team at the TOXALIM research center in food toxicology (Toulouse, France). His research focuses on the metabolism of food and environmental contaminants, with an emphasis on the relationship between specific bioactivation pathways and adverse effects. He has been involved for 20 years in studies related to endocrine disrupting chemicals (EDCs), investigating the relationship between biotransformation pathways and toxicological endpoints. He will chair the 2018 GRC conference on EDCs. Among others, the team published the first study documenting the fate of bisphenol A (BPA) at low doses over the perinatal period (2003), demonstrated the passage of BPA through skin in humans (2010) and showed that halogenated bisphenols and their metabolites are PPARg agonists (2014). In parallel, the Metabolism of Xenobiotics team has pioneered the use of global approaches in the field of toxicology. A unique pipeline was developed for global approaches (metabolomics) dedicated to the study of the effects of EDCs. Proofs of evidence that NMR based fingerprints could be used as powerful tools to discriminate between control and exposed biological systems were provided in vitro (2010) and the team released the first in vivo metabolomics study related to EDCs (perinatal exposure of rodents to BPA, 2013), highlighting lasting effects on the F1 generation born from dosed dams. The team, which gathers researchers in bioinfomatics and toxicologists, developed a specific analytical and bio-informatics pipeline including the Metexplore webserver (http://metexplore.toulouse.inra.fr/) to address these questions, with the aim to decipher the mechanisms of action of low doses of EDCs through unbiased (untargeted) global approaches, a current key scientific issue in the field of toxicology.

Metabolomics and systems biology in studies on endocrine disruptors

Twenty-five years have passed since a small group of scientists first proposed the hypothesis that anthropogenic substances could behave as endocrine disrupting chemicals (EDCs), with significant impacts on the health of wildlife species, but also of human beings. Over the next decades, major controversies in the field of EDC research emerged in the context of a growing awareness of the public regarding the consequences of food and environmental contaminants exposure. This includes debates about low-dose and non-monotonic effects, perinatal imprinting and additive effects of EDCs. Not so surprising was the gradual discovery that EDCs targets largely exceed the framework of reproductive effects. Indeed, the endocrine system regulates the whole homeostasis of living organisms, and sharply tunes processes from development and growth to general metabolism. Advances in the field of metabolomics (and other omics approaches) have opened new perspectives for toxicologists exploring EDCs effects. Metabolome's modulation faithfully reflects the consequences of internal biochemical changes. Solid evidence was provided since 2013, demonstrating that "metabolic fingerprints" can successfully discriminate exposed animals and unveil early biomarkers of perinatal imprinting by chemicals. Convincing evidence in humans was also provided by few studies, despite difficulties linked with the high variability of both human genetics and exposure conditions.

Newest results even suggest that some EDCs could have transgenerational effects. Metabolomics have as well been successfully applied to in vitro systems which use is rapidly growing in toxicology. Exceeding this descriptive approach, current advances in systems biology open new roads to better understand the effects of chemical pollutants. The modelling of "metabolic networks", based on multi-omics data, and the study of its shifts under contaminants exposure, is expected to provide valuable data on EDCs modes of action, help seek predictive biomarkers, and assess whether characteristic metabolic shifts should be considered as mere adaptive changes or as adverse effects.

FRANCIS L. MARTIN

Pharmacy and Biomedical Sciences, University of Central Lancashire (Preston, UK).

Prof. Francis L. Martin PhD FIBMS (FLM) leads a world-leading laboratory in the emerging field of spectrochemical analysis increasingly known as biospectroscopy. His group has pioneered the application of these techniques in a diverse range of areas, including cell biology, biomedical, plant physiology, environmental science and toxicology. He is currently Biosciences theme lead at University of Central Lancashire (UCLan). Critically, his group has led inter-disciplinary developments including computational handling of complex datasets and surface-enhanced Raman spectroscopy (SERS); this is evidenced by the number of publications (>200) from his group published in journals including Nature Protocols, Analyst, Proceedings of the National Academy of Sciences USA and ACS Nano. FLM's group has hosted many visitors from within the UK and internationally. There is increasing recognition that these biospectroscopy techniques will have a diverse range of end-user applications, both to address academic questions and for applied measurements, e.g., screening and/or diagnostic assessments for clinical applications. Prior to his current position at UCLan, FLM gained his PhD at University College London, was a post-doctoral Research Fellow at Institute of Cancer Research (UK) for >6 y and established his laboratory at Lancaster University over 15 y. He has been President of the UK Environmental Mutagenicity Society (UKEMS) and sits on the Department of Health Public Health Service Committee of Experts on Mutagenicity of Chemicals in Food, Consumer Products and the Environment. Additionally, he has had significant past and current industry collaborations with Unilever and is co-founder of a spin-out companies, ReVivoCell and Biocel. In 2014, FLM organised and hosted at the biennial European Environmental and Mutagen Society (EEMS) conference (with >300 registrants), which was followed by the industry-focused ILSI/HESI workshop

Efficacy of spectrochemical biomarkers to identify sublethal stress in environmental settings

Worldwide amphibian and other sentinel populations are declining due to habitat loss, disease and pollution. Vulnerability to environmental contaminants such as pesticides will be dependent on the species, the sensitivity of the ontogenic life stage and hence the timing of exposure and the exposure pathway. In a series of studies, we focused on the Common frog, Rana temporaria, and employed spectrochemical analyses to investigate the biochemical tissue 'fingerprint' in spawn and early-stage tadpoles from urban and agricultural ponds with contrasting water quality. Tissue analysis using attenuated total reflection-Fourier-Transform infrared (ATR-FTIR) spectroscopy revealed only subtle differences in biochemistry between spawn from the different ponds. For tadpoles of the same Gosner life stage, gross morphological differences (e.g., head width and Snout-to-Vent length) were also not apparent between the ponds. However, marked differences ($p < 0.05$) were observed in the ATR-FTIR spectra between tadpoles from a rural agricultural pond with no pesticide input and those from both an agricultural pond impacted by pesticides and an urban pond affected by wastewater and landfill run-off. These differences related principally to carbohydrates, particularly the glycogen region of the spectra (1030-1150 cm-1) and to a lesser extent the phosphate chain vibrations in nucleic acids and phospholipids (e.g., 1003 cm-1). Tadpoles from the intensive agricultural and urban ponds have altered levels of glycogen in comparison to those from the rural agricultural pond. In the absence of population surveys our results demonstrate that levels of stress (marked by biochemical constituents involved in compensatory metabolic mechanisms) can be observed in tadpoles in freshwater systems with low water quality. These observations in amphibians and other sentinel organisms (e.g., sparrowhawk) point to a critical role of environmental pollutants in generating pathogenic effects and that spectrochemical analyses can play a central role in identifying and monitoring such effects.

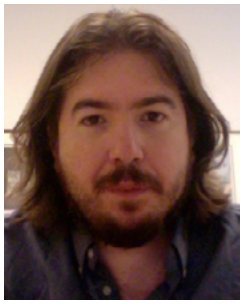
AGE SMILDE

Biosystems Data Analysis, Swammerdam Institute for Life Sciences, University of Amsterdam (Netherlands).

Age K. Smilde is full professor of Biosystems Data Analysis at the Swammerdam Institute for Life Sciences at the University of Amsterdam and as of June 1, 2013 he also holds a part-time position as affiliate professor at the Department of Food Science at the University of Copenhagen. He has published more than 230 peer-reviewed papers and has been the Editor-Europe of the Journal of Chemometrics during the period 1994-2002. He is a co-founder of the Netherlands Metabolomics Centre; a large Public/Private Consortium devoted to all aspects of metabolomics His research interest is data fusion and multiset methods. For more information: see www.bdagroup.nl

Common and distinct components in data fusion

One of the active research topics in data fusion is to separate common from distinct variation in multiple blocks of data. This goes under different names such as shared/unshared variation, joint/individual variation and unique/specific variation. We will present a mathematical and geometrical framework for common and distinct variation and give definitions to structure the field. Some methods will be placed in that framework and worked out in several examples of metabolomics data. Of special interest is the extension of these methods to data of different measurement scales, such as ratio-scaled and binary data. These type of data are increasingly becoming available in genomics. We will present some initail ideas and examples.



FEDERICO MARINI

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Dr Marini received his MSc from Sapienza University of Rome in 2000 where he also completed his PhD in 2004. He was Marie Curie fellow at the National Institute of Chemistry (Ljubljana, Slovenia) in 2003. Since 2008 he is researcher and professor of Chemometrics at Sapienza University of Rome. In 2006, he was awarded the Young Researcher Prize from Italian Chemical Society and in 2012 he won the Chemometrics and Intelligent Laboratory Systems Award “for his achievements in chemometrics”. He has been visiting researcher in various Universities (Copenhagen, Stellenbosch, Silesia, Lille). His research activity is focused on all aspects of chemometrics, ranging from the application of existing methods to real world problems in different fields to the design and development of novel algorithms. He is author of more than 85 papers in international journals, and recently he edited and coauthored the book Chemometrics in food chemistry (Elsevier). He is member of the Editorial boards of Chemolab, Analytica Chimica Acta, J. of NIR Spectroscopy, J. of Spectral Imaging and he serves as Associate Editor for Chemometrics in Wiley’s Encyclopedia of Analytical Chemistry. He is currently the coordinator of the Chemometric group of the Italian Chemical Society and a member of the Chemometric study group of EUCheMS. He’s also recently been appointed extraordinary professor at the University of Stellenbosch for the years 2017-2020.

Approaches for the analysis of designed metabolomic data

In many metabolomics studies, one may be interested to investigate or understand the effect of some process or intervention (the factors) on one or more properties (the responses) of a system: in all such cases, to obtain the maximum desired information from the recorded data, the use of a rational design of the experiments to be carried out is necessary. On the other hand, considering that the resulting outcomes are affected by the presence of multiple sources of variation, data coming from an experimental design call for suitable statistical approaches for providing the desired information. In this context, whereas in the case of univariate or oligovariate responses, well established methods for the evaluation of the effect of the controlled factors and their interactions exist in the framework of the theory of linear models, the classical multivariate analysis of variance becomes inapplicable when the number of experiments is insufficient compared to the number of responses. To overcome this limitation, during the last years different approaches have been proposed for the analysis of multivariate data coming from designed experiments, among which ANOVA-PCA, ANOVA-simultaneous component analysis (ASCA) and ANOVA-target projection could be listed.

In the present communication, the main characteristics of the different methods proposed for the multivariate analysis of designed data will be discussed, and attention will be particularly focused on stressing the differences and similarities among them, also by means of real world examples.

JOHAN TRYGG

Kemiska institutionen Umeå universitet, (Umeå, SE).

Professor in Chemometrics who pioneered the development of multivariate analysis methods aimed at improving interpretation, data integration and visualization of large complex multivariate data. This includes the Orthogonal Projections to Latent Structures (OPLS) solutions and its extensions, OPLS-DA, O2PLS and OnPLS, already in use by more than 150 companies, 50 international institutions and 23 global pharma companies. Johan received his Ph.D. in 2001, in Chemometrics at Umeå University, Sweden (Prof. Svante Wold). Following a postdoc in bioinformatics at Univ. Queensland, Australia, and in metabonomics at Imperial College, London, he returned in 2003 to Umeå University where he became full professor in chemometrics in 2012. Now Johan is heading the Computational Life Science Cluster, a bioinformatics platform, and is vice platform director for the Swedish Metabolomics Centre (http://swedishmetabolomicscentre.se/). Since 2015, Johan is also a Visiting Professor at Imperial College and Senior Director of R&D at Umetrics (now Sartorius Stedim Data Analytics).

Johan is a frequently invited speaker at international conferences. Scientific track record includes 145 publications, H-factor (40) and six granted patents and several awards. He currently serves as Chair of the Chemometrics chapter, Swedish Chemical Society and was the Associate Editor for Journal of Proteome Research during 2015-2016.

Multivariate analysis and integration of multi-OMICS data

Today, large amounts of experimental data are being generated by modern high-throughput ‘omics’ technologies. The possibility to characterize a single sample using several profiling techniques generates multiple sets of large complex data. Extracting and integrating useful information from these data sets is a nontrivial task.

Here, descriptive data analytics by means of the OnPLS approach can be used to quantitatively describe the main features in a collection of datasets and effectively map and visualize how different types of variation in each dataset is connected with the others. It can also provide the unique variation each individual dataset holds that is not found in the other datasets. More specifically, OnPLS decomposes each dataset into a globally joint part containing variation shared with all other connected data sets, a locally joint part containing variation that is shared with some, but not all, other data sets and unique variation, present only in a single data set.

We will demonstrate the OnPLS capability for data integration of complex multi-omics datasets for enabling biological understanding of the studied systems.

ANA CONESA

Intituto Principe Felipe (Valencia, Spain).

I lead the Genomics of Gene Expression Lab at the Computational Genomics Program of the Centro de Investigaciones Príncipe Felipe, Valencia (Spain) and I am Professor of Bioinformatics at the Microbiology and Cell Science Department at the University of Florida in Gainesville. I graduated as Agricultural Engineer at the Polytechnical University of Valencia in 1993 and did my PhD in Molecular Microbiology at the University of Leiden in the Netherlands. After a short appointment as bioinformatics project leader at TNO Quality of Life (The Netherlands) I obtained a Ramon y Cajal award and joined the Valencia Agricultural Research Insitute in 2003. I moved to CIPF in 2007 and I became Senior Group Leader in 2010 and UF Professor in 2014. I am interested in understanding functional aspects of gene expression at the genome-wide level and across different organisms. My group has developed statistical methods and software tools that analyze the dynamics aspects transcriptomes, integrate these with other types of molecular data and annotate them functionally, with a special focus on Next Generation Sequencing (NGS) data. Some of our popular software tools are Blast2GO, Paintomics, maSigPro, NOISeq, Qualimap, SQANTI, etc. I have published over 90 research papers that have received more than 12000 citations. I have participated in numerous EU projects, and I coordinted two of them. The STATegra project on multiomics data integration, and the Marie Curie Action DEANN for creating a NGS network with American countries. I am co-founder of Biobam, a bioiformatics company that commercializes user-friendly software for biologists. My current research interest is the development of statistical methods for multiomics data integration and the creation of tools for the analysis of third-generation sequencing data.

Multiomics data integration to unravel local gene regulatory networks

The omics technologies have evolved over the last two decades to offer an array of platforms that are able to measure different types of molecular features on a cell-wide scale. The combination of several of these methods has been instrumental in profiling mechanisms that link gene expression with other regulatory layers and we are currently witnessing a dramatic increase in the number of studies where multiple omics technologies are combined. The multiomics approach aims at providing a more complete description of cellular components and reveal systems interactions that cannot be fully understood using only one type of measurement. Moreover, the integration of biomarkers from multiple molecular layers may potentially increase our capacity for developing tools for precision medicine.

However, while the multiomics approach is becoming a common strategy in genomic research, there is still a lack of clear guidelines to address the particular problems of multiomic projects. Experimental design, integrative analysis and visualization of multiomic data pose a number of challenges that were not present in studies where only one type of measurement was considered. For example, are sample size requirements the same across omics platforms? how do we deal with many-to-many possible relationships among omics features? how does the coverage of the feature space in each technology compare and how does this impact analysis? how do we represent data where chromatin and pathway level information needs to be combined? Motivated for these questions and the lack of software and guidelines for the analysis of multiomics data the STATegra project was develop. In my talk I would present STATegra efforts to propose a road map for multiomics projects. We highlight six different steps involving experimental design, pre-processing and explorative analysis, matching omics features, statistical methods for data integration to define gene expression regulatory models, visualization and validation. At each step, I will present specific solutions developed by the STATegra Multiomics Data Analysis Project. We show case these methods using a controlled experimental system mimicking a defined stage in the differentiation of the B-cell lineage in mouse and created a time course multiomics dataset comprising up to 8 different platforms. I hope this presentation will provide lights on the complexity, the possibilities and the existing challenges for applying multiomics approaches to solve basic and practical biological questions.

MARTA CASCANTE

Department of Biochemistry and Molecular Biology, Facultat de Biologia, Universitat de Barcelona (Barcelona, Spain).

Marta Cascante is Full Professor at the Department of Biochemistry and Molecular Biology at University of Barcelona and leader of the Integrative Systems Biology, Metabolomics and Cancer team. She has authored over 200 publications, two of them cited in "Stryer" biochemistry textbook. She is partner of European translational research projects (H2020) in the field of systems medicine and metabolomics and member of the editorial advisory boards of Metabolomics and BMC systems Biology. She has been distinguished in 2015 with Icrea Academia Prize and with the Narcis Monturiol Medal of the Catalan Government for her scientific merits.

Research interests are focused on cancer and metabolic diseases with the goal of elucidating the networks and pathways that are fundamental in their development and progression. More specifically, her team uses a Systems Medicine approach to identify key proteins in the metabolic reprogramming underlying multifactorial diseases, including cancer, to be used as biomarkers or drug targets. In the coming years her team plan to develop a multi-omic approach to construct genome-scale metabolic networks that accurately reflect short-term and long-term metabolic adaptations associated with metastasis and acquired therapeutic resistance. Furthermore, in the framework of H2020 project "PheNoMenal ", coordinated by EBI, her team is contributing to develop and deploy an e-infrastructure that makes it feasible for healthcare researchers to process analyze and mine molecular phenotyping data, to facilitate large-scale data analysis in the coming age of Precision Medicine.

A systems medicine approach to target metabolic reprogramming associated to metastasis

Progression towards metastasis is initiated by a cellular process known as epithelial-mesenchymal transition (EMT) and followed by the participation of a minority of malignant cells known as tumor-initiating cells (TICs). Using a well characterized clonal prostate neoplastic cell subpopulations displaying stable and distinct EMT (epithelial-mesenchymal transition) or e-CSCs (metastatic epithelial stem cell) phenotypes, and combining metabolomics, 13C-fluxomics and genome scale metabolic network reconstruction approaches, we have characterized differential key features in their metabolic reprogramming with potential clinical implications. Moreover, analysis of transcriptomic data yielded a metabolic gene signature for our e-CSCs, consistent with the metabolomics and fluxomics analysis that correlated with tumor progression and metastasis in prostate cancer and in 11 additional cancer types, suggesting potential biomarkers and therapeutic targets to effectively forestall metastasis.

Finally, workflow for 13C-fluxomics (in development in the framework of the e-infrastructured project PhenoMeNal www.phenomenal-h2020.eu/home/) to facilitate the use of existing 13C-fluxomics tools and metabolomics data repositories as MetaboLights (www.ebi.ac.uk/metabolights) is also presented.

LARS KELD NIELSEN

Professor & Scientific Director of Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark (Lyngby, Denmark), Professor & Chair of Biological Engineering Australian Institute for Bioengineering and Nanotechnology (AIBN) University of Queensland, Brisbane Qld 4072, Australia

Professor Nielsen's core interest is modelling of cellular metabolism. His team has made many contributions to the formulation and use of genome scale models and produced the first mammalian and the first plant genome scale models. Professor Nielsen heads the BioPlatforms Australia Queensland Node for Metabolomics and Proteomics focusing on developing quantitative analysis to support metabolic modelling. He recently received a Novo Nordisk Foundation Laureate Research Fellowship to develop large scale, accurate kinetic models of metabolism.

Kinetic modelling within our GRASP: new strategies for fitting accurate network kinetic models

Large scale kinetic models of metabolism are required to explore and explain the molecular basis for homeostasis, the self-regulating processes evolved to maintain metabolic equilibrium. Studying homeostasis is relevant for the understanding and treatment of complex diseases, particular with the emergence of personalized medicine. It is equally important when we seek to repurpose the cellular machinery for the production of desired chemicals, materials and pharmaceuticals. In the latter process, the cells' homeostatic control mechanisms must be either disabled or exploited. However, estimation of in vivo parameters is hard due to the large amount of data needed and the fact that homeostatic control typically renders many parameters practically unobservable. We have developed a General Reaction Assembly and Sampling Platform (GRASP) capable of consistently parameterizing and sampling thermodynamically feasible kinetics using minimal reference data (Saa & Nielsen, PLoS Comput Biol 11, e1004195, 2015). The former integrates the concerted MWC model and elementary reaction formalism to describe both simple and complex (e.g., allosteric) kinetics. Application of this approach enabled assessment of the impact of thermodynamics on reaction kinetics, as well as the exploration of complex allosteric behaviours (Saa & Nielsen, BBA – Gen Subjects 1860, 576-587, 2016). Formulation of the sampling problem within the Bayesian framework provides a natural interface for the addition of experimental data, hereby improving sampling accuracy. Approximate Bayesian Computation (ABC) methods were used to unravel the dynamic properties of metabolic networks (Saa & Nielsen, Sci Rep 6, 29635, 2016). A posteriori analysis of the parameter distribution enabled prediction of metabolic states, identification of critical parameters as well as unravelling the control structure of the network. We have illustrated the capabilities of our framework in several cases ranging from simple enzymatic mechanisms to tightly-regulated pathways. Overall, this framework demonstrates that detailed kinetic representation is possible without sacrificing complexity and with low amounts of data. Expanding this framework to large metabolic networks requires further algorithmic advances, including the development of efficient and robust sequential sampling schemes. Recent advances in algorithmics will be discussed.

ALEX SÁNCHEZ-PLA

Statistics and Bioinformatics Unit of the Research Institute "Vall d'Hebron Institut de Recerca"

I graduated in Biology (1980) and obtained a Ph.D. in Statistics (1996), at the program of Probability and Statistics, Universitat de Barcelona. I studied mathematics at the Universidad Nacional de Educación a Distancia (UNED) and I obtained a Master in Bioinformatics (2005) by the University of Manchester. I am currently Professor ("Titular de Universidad") in the Department of Genetics Microbiology and Statistics, Universitat de Barcelona. I am also the Head of the Statistics and Bioinformatics Unit (UEB, http://ueb.vhir.org) of the Research Institute "Vall d'Hebron Institut de Recerca"(www.vhir.org) . I am a member of several scientific societies and have served as president of the Spanish Region of the International Biometric Society. My research interest lies in the interface between Statistics and Bioinformatics. Specifically I am interested at developing and applying methods and tools for the integrative analysis of Omics data. I participate in collaborative researchs in biomedical studies in neurosciences, immunology, oncology and nutrition and metabolomics. I am group leader of the Statistics and Bioinformatics Research Group (http://eib.stat.ub.edu) now embedded in the GRBio (Research Group in Biostatistics and Bioinformatics) UPC-UB group (http://grbio.eu). Recently we have started a fruitful collaboration with the UB's Biomarkers and Nutritional & Food Metabolomics research group (www.nutrimetabolomics.com) which has shifted part of our efforts onto this promising field. My teaching activities are also related with these disciplines. I teach Advanced Statistical Inference, at the Msc of Statistics and Operations Research (UB-UPC), and Omics Data Analysis at the same master. I am currently University of Barcelona coordinator of the joint UOC-UB online Msc of Bionformatics and Biostatistics.

An equivalence test approach to the integrative analysis of gene lists

The analysis of lists of features (genes, proteins, microRNAs etc.) has been an active field since the appearance of omics data analysis. Its main application has been in the development of methods and tools for biological significance analysis -Over Representation Analysis and Gene Set Enrichment Analysis being the best known-but also the development of gene sets and signatures that have been applied for classification or prognostic purposes. One topic that has received less attention is the comparison of gene lists from the point of view of their biological meaning. This seems a relevant problem, especially in the post-genomics age, where multiple datasets from the same or different type, are available for study. This can be used for example, in data fusion problems, in data mining of gene signatures or in a meta-analysis context. We discuss here extensions of the goProfiles approach, a method for comparing gene lists relying on their functional representation which is based on taking lists of genes and projecting them into predefined levels or slices of the Gene Ontology, in such a way that a multinomial model can be used for estimation and testing. Although the method has been built in a classical hypothesis testing context we present here an approach more suited to integrative analysis where the main interest is in establishing -not in rejecting- the similarity between two lists. With this aim in mind we have derived an equivalence method which uses a distance–based approach and the confidence interval inclusion principle. In this talk we show how equivalence tests developed for one pair of lists can be naturally extended to establish the equivalence of any number of gene lists by taking an iterative approach that combines a bottom-up approach to determining the most to least equivalent gene lists while adjusting for multiple testing.

ORAL COMMUNICATIONS

SESSION 1: GENOMICS AND TRANSCRIPTOMICS

METHYLATION SENSITIVE HIGH RESOLUTION MELTING AS A HIGH THROUGHPUT TECHNIQUE TO DETECT DNA METHYLATION ALTERATIONS AFTER EXPOSURES TO ENDOCRINE DISRUPTORS

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Abstract

Environmental insults occurring during the early development may have life-long effects. While different epigenetic mechanisms are assumed to mediate these effects, the precise modes of action are largely unknown and the application of these approaches in ecotoxicology is still lacking. Zebrafish is a recognized model for the analysis of epigenetic mechanisms in developmental biology and in environmental epigenetics, thanks to the similarities in epigenetic regulatory machinery in all vertebrates. Here we apply the Methylation Sensitive High Resolution Melting (MS-HRM) methodology to evaluate changes in methylation patterns of specific promoter regions in zebrafish embryos exposed to 4ppm of BPA during the first five days after fertilization. For this study, we used data from a parallel study that characterizes the effects of BPA by simultaneous transcriptomics and metabolomics analyses. These results showed changes in genes and metabolites related to response to estrogen stimulus, lipid transport and metabolism, retinol metabolism and PPAR signalling pathways, among others. Consistently, we selected two genes implicated in the estrogen and the retinoid signaling pathways to analyze DNA methylation profiles of their promoters. The aldehyde dehydrogenase 1 family member A2 (aldh1a2) codifies for an enzyme that catalyzes the synthesis of retinoic acid from retinaldehyde, and the gene becomes activated upon addition of pure retinoids. Similarly, the cytochrome P450 family 19 subfamily A Member 1b (cyp19a1b) codifies for an enzyme that catalyzes the last steps of estrogen biosynthesis in zebrafish brain, and the transcription of the gene increases upon the addition of exogenous estrogens. Differential melting profiles were detected by MS-HRM in specific regions of both selected gene promoters after BPA exposure, suggesting alteration of methylation patterns. These results are being corroborated by bisulfite sequencing of the same promoter regions. We anticipate that MS-HRM will be a powerful technique to perform high throughput analysis on the effects of many EDCs on DNA methylation patterns.

Keywords - BPA, DNA methylation, zebrafish, EDCs, methylation-sensitive high resolution melting

SESSION 1: GENOMICS AND TRANSCRIPTOMICS

DEVELOPMENT OF MICROBIAL INDICATORS OF ANAEROBIC DIGESTION INHIBITION WITH OMICS

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Abstract

Anaerobic digestion (AD) is a complex microbiological process of degradation of the organic matter which produces biogas rich in methane. It is used to manage different types of waste and produce energy at industrial scale. However, AD is not fully controlled and exploited, mainly because of the absence of microbial-based management tools. Omics methodologies now enable to deepen the knowledge of AD microbiota and pave the way to the development of such tools.
Our work was focused on AD inhibition, a recurrent operational issue. Sixty lab-scale anaerobic batch toxicity assays were conducted in parallel (triplicate) with ten concentrations of phenol and ten concentrations of ammonia, two well-known inhibitors. Influence of the inhibiting conditions on AD performance was described. It ranged from no inhibition to total failure for both inhibitors. Samples were taken regularly to characterize associated microbial dynamics through the sequencing of both 16SRNA and DNA 16S gene (144 samples). Obtained datasets were statistically analysed with mixOmics R package to identify biomarkers of the inhibition.
Several hundred types of microorganisms were identified in the samples. RNA and DNA datasets, compared with canonical correlation analysis, enabled a rather similar classification of the samples. For both genomic and transcriptomic data, classical multivariate methods (PCA, MDS, NMDS) showed a distinct influence of inhibition level and type of inhibitor on microbial composition. Partial Least Square-Discriminant Analysis (PLSDA) enabled to identify several microorganisms signature of phenol or ammonia inhibition (8 with DNA, 10 with RNA). With Partial Least Squares Regression (PLS), other microorganisms specific of low or high inhibition levels, independently of the type of inhibitor, were identified. Temporal analysis showed that microbial shifts began before AD performance was seriously impaired by the inhibitors. We thus believe that the identified microorganisms could be used as early-warning bioindicators to prevent digester failures induced by inhibition.

Keywords - complex microbial community, sequencing, biomarkers

SESSION 1: GENOMICS AND TRANSCRIPTOMICS

TRANSCRIPTOMIC RESPONSES TO TEMPERATURE SHIFTS IN A MEDITERRANEAN KEYSTONE SEA URCHIN

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Abstract

Arbacia lixula is a sub-tropical sea urchin that colonized the Mediterranean Sea during the last interglacial period, and it is among the main marine barren-forming species in that Sea. Although it lives at suboptimal temperatures in the Mediterranean, recent investigations suggested a positive effect of global warming on its reproduction success, and hence a worrisome increase in its abundance. In the present study, we explore the molecular machinery involved in the response to thermal stress in A. lixula to understand its potential performance under temperature shifts. Since the magnitude of the transcriptomic responses can be used as a measure of the organism performance, we quantified gene expression using RNA-seq techniques in thermal stress assays. We exposed sea urchins to three different treatments (6 specimens per treatment) and set two different experiments for 20 hours: a "cold experiment" including 13°C (control) versus 7°C; and a "warm experiment" including 13°C (control) versus 22°C, and we then compared transcriptomic patterns between treatments and experiments using the de novo assembly of A. *lixula* transcriptome as a reference. For the "cold experiments", 1,181 transcripts were differentially expressed (DE) (adjusted p-value < 0.01), being 720 up-regulated and 461 down-regulated at 7°C. Out of the 1,181 DE transcripts (adjusted p-value < 0.01), only 445 (~37.7 %) had a known function. For the "warm experiment" only 179 transcripts were DE, being 57 up-regulated and 122 down-regulated at 22°C. Out of the 179 DE transcripts, only 35 (~19.7 %) had a known function. Fourteen DE transcripts were found in common between experiments, but most of them had opposite responses. Our results demonstrate a much higher gene expression response to cold temperatures in A. *lixula*, hence increasing the concern about the potential negative impact of the species due to a better performance under a scenario of global warming.

Keywords - gene expression, stress, climate change

SESSION 2: METABOLOMICS AND LIPIDOMICS

METABOLOMICS REVEALS METABOLIC DISORDERS IN MICE EXPOSED TO THIRDHAND TOBACCO SMOKE

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Abstract

Thirdhand tobacco smoke (THS) is a novel and poorly understood pathway of tobacco exposure that is produced by the deposition and ageing of tobacco smoke particles and toxicants in surfaces and dust. This aged tobacco smoke becomes increasingly toxic with age, re-emitted into the air or react with other chemicals in the environment to yield new toxicants, including carcinogens. Furthermore, THS remains in indoor environments long after smokers move out, which makes THS a serious health problem, especially for children with smoking parents. Although the increasing evidences of THS hazards, the specific cellular and molecular consequences of exposure to THS remain to be fully elucidated. Here we present the first non-targeted metabolomics approach applied to THS-exposed animal model: C57BL/6 mice, exposed to THS under conditions that mimic exposure of humans in homes of smokers. THS-exposed mice showed alterations in multiple organ systems including non-alcoholic fatty liver disease, inflammation in the lung, hyperactivity, hyperglycemia and insulin resistance through oxidative stress. Urine samples from THS-exposed mice and control ones were analyzed using GC-QTOF and reverse phase HPLC-QTOF in both ionization modes. Accurate multivariate analysis revealed hundreds of differently expressed metabolites, including several of the tryptophan metabolism. Interestingly, some of the altered metabolites coincide with those reported in metabolomics studies of current smokers. Nevertheless, this study also reveals for the first time other metabolic pathways altered by this passive way of tobacco exposure, not related to tobacco until now. Furthermore, biomarkers of tobacco exposure have been also detected in the urine of the THS-exposed animals using a target HPLC-QQQ approach in concentrations similar to those reported in children exposed to second hand smoke.
This study demonstrates the health hazards of THS exposure and, if confirmed in humans, would have a major impact on current tobacco health and environmental policies.

Keywords - Environmental exposure to tobacco; Thirdhand tobacco smoke; multiplatform metabolomics.

SESSION 2: METABOLOMICS AND LIPIDOMICS

RE-THINKING ECOTOXICOLOGY PARADIGMS IN THE OMICS ERA

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Abstract

As technologies advance, the expectation is that we will become better equipped to understand the various ways that anthropogenic stressors are influencing the environment. When paired with standard toxicity bioassays, environmental omics (e.g., genomics, proteomics, and metabolomics) provide a wealth of information related to sub-lethal effects. However, the scope of omics data can also reveal sources of variance and confounding not readily apparent with traditional test endpoints. Considering the mounting concerns regarding irreproducibility in science, omics techniques should therefore also be viewed as an opportunity to critically evaluate and improve upon fundamental approaches used for ecotoxicology research. For example, standard bioassay protocols commonly use organic carrier solvents such as methanol to improve chemical dispersion and solubility, as a means of achieving target concentrations and establishing dose-response relationships. Recommended maximal solvent doses have been devised based on evidence of no effect on traditional 'apical' endpoints, but the suitability of carrier solvents when assessing sub-lethal physiological and metabolic pathways is unknown. Direct solvent toxicity is important, but an even greater question exists – are sub-lethal responses influenced by interactions between solvents and contaminants(s) of interest?
The present study applied untargeted 1H NMR-based metabolomics to investigate whether interactions occur between a common carrier solvent (methanol) and a pharmaceutical mixture. Results demonstrate considerable interactive toxicity, with differential metabolite response profiles between solvent and solvent-free approaches to chemical dosing. This could have profound implications for the broad field of aquatic toxicology, since it suggests that the widely accepted paradigm of using organic carrier solvents for chemical dosing may lead to erroneous conclusions about sub-lethal effects.

Keywords - NMR spectroscopy, amphibian development, pharmaceutical, toxicity, mixture, metabolomics

SESSION 2: METABOLOMICS AND LIPIDOMICS

ENVIRONMENTAL EFFECTS OF CYANOBACTERIAL BLOOMS ON FISH: A METABOLOMIC APPROACH FROM EXPERIMENTAL FISH TO NATURAL POPULATION

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Abstract

Cyanobacterial blooms are responsible of the production of a wide variety of potentially toxic secondary metabolites that can impacts on the stability and the functioning of aquatic ecosystems, microcystins (MCs) and other cyanobacterial metabolites have been suspected to induce various negative effects on various fish species.
Recently, with the development of "Omic" sciences, proteomic and metabolomics analyses, using nuclear magnetic resonance (NMR) and/or mass spectrometry (MS), have been shown to provide a powerful tool for the discrimination of metabolic responses between organisms exposed to different treatments and for the identification of metabolic pathways involved in toxicological mechanisms of toxic compounds. However to our knowledge, no such investigations dealing with the global metabolic effects of toxic cyanobacterial blooms have been yet performed on fish.
In this way, liver metabolome profiles were analyzed on four typical fish species of freshwater European lakes (perch, roach, crucian carp and pumpkinseed sunfish) exposed or not to cyanobacterial blooms, and originating from both field enclosures, mesocome experiments and field sampling. The aims of this study are (1) to investigate and identify the metabolic changes which may be observed in the different fish species according the presence of cyanobacterial blooms; (2) to assess and identify the potential different responses of the different fish species metabolomes under stress conditions due to a cyanobacterial exposition. These results originating from multi-approach observations will help us to better understand the genuine ecotoxicological effects of cyanobacterial blooms producing a wide variety of potential noxious metabolites on fish populations from natural ecosystems.

Keywords - Cyanobacteria, fish, ecotoxicology, metabolomic

SESSION 2: METABOLOMICS AND LIPIDOMICS

HYBRID TARGET/NON-TARGET PROFILING OF BIOLOGICAL SAMPLES FOR HUMAN METABOLOMICS & EXPOSOMICS STUDIES

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Abstract

The “omics” world is moving in the direction of exposomics, which can be viewed as metabolomics augmented by a study of exogenous compounds and their metabolites. The central idea is that while metabolomics can aid in understanding how an organism functions and disease states of the organism, to truly explain many diseases and conditions, one must include the burden of exogenous compounds to which an organism has been exposed. Considering that more than 130 million organic and inorganic chemicals were registered to the CAS Registry database, with more than 15,000 new chemicals being added daily, and that these compounds may be present at very low concentrations in the body, this is a daunting task. However, many of the organic compounds that make up the “exposome” are small molecules which are amenable to GC analysis (e.g.: petroleum-based compounds, flavours, fragrances, pesticides, PCBs, PCDD/Fs, etc.). As a platform, comprehensive two-dimensional gas chromatography (GC×GC) with a time-of-flight mass spectrometer (TOFMS) is a nearly ideal tool for studies of metabolomics and exposomics, it offers improved sensitivity over one-dimensional methods, improved separation capacity, leading to cleaner spectra of compounds for identification, minimal matrix effects (when compared with LC-MS), and a broad dynamic range. However, the greatest advantage is that the technique “sees all” with the MS by capturing full mass spectra at every point. Thus it opens the door to hybrid target/non-target techniques. Presented here is such a method for biological samples (urine, plasma, feces, etc) which allows for the quantification of known target metabolites and exogenous samples, while simultaneously detecting unexpected compounds in the sample. This provides a significant advantage in the realm of exposomics where one may not know what they are looking for a priori.

Keywords - GC×GC-TOFMS, Exposomics, Metabolomics, Urine, Blood, Plasma.

SESSION 2: METABOLOMICS AND LIPIDOMICS

IDENTIFYING MARKERS OF LUNG CANCER AND CARDIOVASCULAR DISEASE WITH ADDUCTOMICS

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Abstract

Studies have indicated the potential link between exposure to air pollution and increased risks of diseases such as Lung Cancer or Cardiovascular disease. OMICs measurements (metabolomics, proteomics, etc) are useful tools for the investigation of pathways that lead to the increased risk. Among the OMICs technologies, untargeted adductomics is a new approach that allows the analysis of adducts of human serum albumin (HSA) and reactive electrophiles in serum. Electrophiles present in blood are highly reactive species that have been long suspected of causing cancer and other diseases because of their ability to bind DNA and proteins. The challenge for their measurement is due to their very short half-life in the body and their high reactivity. To overcome this challenge it is possible to measure the adducts resulting from their binding to DNA or proteins (Haemoglobin and Albumin). Protein adducts have a longer life span and are present in much higher concentrations (30 mg of HSA and 0.003-0.008mg of DNA per mL of blood). Therefore, they provide more suitable markers to measure exposure to toxic electrophiles but up to now have been very poorly investigated. The Cys34 locus of the Human Serum Albumin (HSA) represents a preferred reaction site for small electrophilic species. Electrophiles generated from environmental exposure to tobacco and/or air pollution can form adducts with Human Serum Albumin Cys34 loci in human serum and become biomarkers of disease. In this study, we present a new method based on untargeted high-resolution mass spectrometry to measure Cys-34 Albumin adducts in human serum. Over 70 adducts of Cys34 HSA were successfully detected in 200 matched cases and controls serum samples. Previously reported as well as novel adducts were identified. Adducts profiles for Lung Cancer and Cardiovascular disease cohorts differed. This work demonstrates that adductomics is a new interesting approach to the detection of biomarkers of exposure and disease and the characterization of the Exposome.

Keywords - New Omic, adducts, human serum albumin, peptidomics, untargeted analysis.

SESSION 3: CHEMOMETRICS

A NEW OMICS FOR THE ENVIRONMENT: ALGAL SINGLE-CELL FINGERPRINTING WITH FLOW CYTOMETRY

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Abstract

The Dutch river delta serves as endpoint of many European rivers that run through industrial areas. Freshwater quality control is therefore highly important. Effect-based monitoring of this quality is gaining considerable interest, but many assays have issues with sensitivity and do not respond linearly to pollutant concentrations. Microscopic single-cell algal analysis shows that species makeup and phenotypes within algal communities do depend strongly on freshwater toxicity. Such analysis is however very time and labour intensive, which is why the mobile Cytosense algal flow cytometer may be very promising for such analyses. This machine provides a comprehensive untargeted fingerprint of the fluorescence of single algal cells, serving as an 'omics' readout of each cell that can be collected to observe the entire algal community within a water sample. We have outfitted this technology with dedicated and novel chemometrics methodology to turn this from a research instrument into a water quality monitor. For this, we extended our method Discriminant Analysis of Multi-Aspect CYtometry data (DAMACY). The DAMACY approach fits a descriptive PCA model on the single cells to compare between subsequently taken samples in a supervised approach. Our environmental data contains many natural rhythms and patterns that are unrelated to toxicity and therefore we first aim to predict these patterns. We then further study faulty predictions and other deviations from these observed patterns as potential 'process faults', i.e. pollution events. We also take an alternative approach, where we study in mesocosms the response of a real freshwater algal community to controlled herbicide toxicity. This together provides an MSPC-based framework for (1) describing normal dynamic patterns in algae, (2) detecting deviations from such patterns and (3) aligning these deviations with 'known' responses for fault detection.

Keywords - Chemometrics, Method Development, Environmental monitoring, algae.

SESSION 3: CHEMOMETRICS

REGULARIZED MANOVA FOR ANALYSIS OF UNTARGETED MULTI-FACTOR METABOLOMICS DATA

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Abstract

Experimental designs of metabolomics experiments often comprise factors such as concentration of a drug or toxicant, time and/or the gender or age of the study organism. Traditionally, multivariate analysis of variance (MANOVA) is used to analyse such multi-factor data. The correlation matrix, which gives the correlation between all pairs of variables in the data, is central to many multivariate statistical techniques including MANOVA. However, the commonly used sample estimator of the correlation matrix performs poorly for high-dimensional data, including metabolomics data. Because of this, MANOVA is not applicable to a typical untargeted metabolomics data set. Currently, this issue is circumvented by assuming that all pairwise correlations between variables are zero. An example of this approach is ANOVA simultaneous component analysis (ASCA). Here, we investigate the combination of MANOVA with a so-called Stein-type shrinkage estimator of the correlation matrix. We show that the resulting regularized MANOVA (rMANOVA) model is essentially a weighted average of the ASCA and MANOVA models. The optimal weight is determined in a data driven fashion. Compared to ASCA, this method assumes that variables can correlate, leading to a more realistic view of the data. Compared to MANOVA, the model is also applicable when the number of samples is (much) smaller than the number of variables. Simulated and real data examples are used to study the performance of rMANOVA for different numbers of samples and variables. The performance is assessed with respect to proper discrimination between the groups of conditions for a specific factor and identification of associated key metabolites. We conclude that rMANOVA is a highly competitive method for analysis of multi-factor metabolomics data.

Keywords - Untargeted metabolomics; experimental design; multivariate analysis of variance; Analysis of variance - simultaneous component analysis; regularized multivariate analysis of variance; high-dimensional data.

SESSION 3: CHEMOMETRICS

URINARY METABOLOME CHANGES IN PRETERM NEWBORNS FED WITH BREAST MILK OR DONOR BREAST MILK

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Abstract

Breastfeeding is the gold standard for the newborn infant nutrition and especially for at risk preterm infants. Beyond nutritional aspects, human milk contains a wide spectrum of bioactive compounds which improves the regulation of the gastrointestinal tract and the immune system, contributing to disease prevention, infant growth, and development. Those aspects are especially critical in premature neonates, then, when own mother's milk (OMM) is not available, the use of donated human milk (DHM) from milk banking endures the optimal feeding of the newborn.
Herewith, we present results from the analysis by Liquid Chromatography–Ultra High Resolution Time-of-Flight Mass Spectrometry (UPLC-TOFMS) of urinary metabolome assessment from 40 healthy preterm infants fed with OMM and DHM aged between 2 and 4 weeks of life. Feature detection, retention time correction, and alignment of signals was performed using XCMS online. Subsequent data processing, including quality control of the data and building of chemometric models, was carried out employing PLS Toolbox and in-house written functions running in Matlab.
A Partial Least Squares Discriminant Analysis (PLSDA) model was calculated obtaining a significant model (permutation test p-value = 0.011) for the discrimination between urinary metabolomics profile in preterm babies fed with OMM compared to those fed with DHM. The metabolic signature has been studied to gain a deeper understanding of the effect of nutrition on the metabolism of the preterm infant
The present study will also include the gut microbiota composition in the recruited patients by use of next generation sequencing (NGS). The combined information about microbiome and metabolome constitutes a systems biology approach that will provide with a better understanding of the role of nutrition in the healthy preterm infant.

Keywords - Breastfeeding, HPLC-TOFMS, Neonatology, Preterm newborn.

SESSION 3: CHEMOMETRICS

HYPERSPECTRAL IMAGING AND CHEMOMETRICS FOR ENVIRONMENTAL – OMICS

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Abstract

The analytical side of environmental –omics is often linked to techniques, such as (LC-, GC-, EC-MS), NMR,… that work with liquid sample extracts and, therefore, involve the destruction of the target organisms to obtain information. Despite the rich qualitative and quantitative information obtained on the metabolome changes, conclusions are inferred at a global organism level and cannot be assigned to effects on particular organs or tissues.

Hyperspectral imaging allows working with physical sections of target organisms and preserves the anatomic morphology of the biological samples. This particular characteristic opens the possibility to test the effect of environmental stressors on model organisms at a specific tissue level. Besides, the spectroscopies available on the diverse imaging platforms provide information on different aspects of the biological constituents of samples.

Handling hyperspectral image information linked to –omic studies goes often through a first study of multisets formed by images from the same population, e.g. control, exposed to stressors. Multiset analysis based on multivariate resolution methods, such as Multivariate Curve Resolution-Alternating Least Squares (MCR-ALS) characterizes these populations by providing spectral signatures and related distribution maps of the biological components of the images, often linked to specific tissues or cell compartments [1]. Although a visual comparison of the shapes of analogous resolved spectral signatures (fingerprints) among populations can already warn about –omic changes at a specific tissue level, MCR output is usually the seeding information for further and more conclusive data analysis procedures. Thus, spectral signatures obtained from MCR-resampled multisets can be employed as input in classification studies (e.g., by PLS-DA) or ANOVA-based methodologies (e.g., ASCA) to assess the effect of environmental stressors at a specific biological component level. Or multiset distribution maps can be used for segmentation purposes [2]. Other options linked to the use of images for –omic studies involve data fusion connecting images from different platforms for a better characterization of the biological components of samples.

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SESSION 4: DATA INTEGRATION AND MODELING

STUDY OF THE EFFECTS OF CHRONIC EXPOSURE TO ALDRIN IN PROSTATE CANCER VIA MULTI-LEVEL APPROACHES

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Abstract

Endocrine disruptors (EDs) trigger molecular events which effects are propagated through the intricate metabolic and regulatory networks, provoking a metabolic reprogramming that ultimately may lead to tumor progression and metastasis. A large number of evidences highlight a dose-effect relation between EDs and tumor progression and malignancy. However our understanding of the effects of chronic exposure to non-lethal concentrations of EDs in cancer metabolism still remains limited.
Here, we have delved into the effects of the chronic exposure to Aldrin (an ED) on the molecular processes in DU145 prostate cancer cells, by combining model-driven and data-driven approaches to integrate multiple omic data.
More specifically we have developed and applied different model-driven methods to integrate metabolomics, lipidomics and transcriptomic data into genome-scale metabolic network reconstructions which has enabled the characterization of key features of the metabolic reprogramming in Aldrin-exposed cells.
Moreover, transcriptomic data analysis based on gene-set enrichment analysis, has unveiled gene-regulatory and signaling pathways associated to the enhanced malignant phenotype in Aldrin-exposed cells. In addition this approach has permitted to find significant correlations between the transcriptomic profiles of the DU145 cells exposed to Aldrin and expression datasets of tumor progression in different tumor types.
Altogether, the use of these complementary approaches provides an overview that permits to better understand the molecular mechanisms underlying the malignant phenotypes observed in Aldrin-exposed cells as well as to find potential relationships with tumor progression and malignancy in different tumor types with important environmental and clinical implications.

Keywords - Omic-data integration, Metabolic modeling, Cancer, Endocrine Disruptor.

SESSION 4: DATA INTEGRATION AND MODELING

REXPOSOME: A BIOINFORMATIC TOOL FOR CHARACTERIZING MULTIPLE ENVIRONMENTAL FACTORS AND ITS ASSOCIATION WITH DIFFERENT OMICS BIOMARKERS AND DISEASE

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Abstract

Exposome encompasses all environmental factors from conception until old age. Due to the ever changing environment and habits, exposure to environmental contaminants is growing increasingly complex. The HELIX 'early-life exposome' approach involves combining all environmental hazards that mothers and children are exposed to, and linking this to the health, growth and development of the children.

The main objectives of HELIX project include the measurement of a wide range of chemicals and physical environmental hazards in food, consumer products, water, air, noise, and the build environment. Also to define a multi-pattern and individual exposure variability while determining a molecular profile and biological pathways associated with those multiple exposures. To this end, a Bioconductor package has been developed. The package incorporates functions for exploring exposome and its interaction with outcomes, its integration with different omic data as well as downstream analyses to facilitate biological insights though pathway analyses and retrieving data from public databases such as DisGeNET and Comparative Toxicogenomics Database.

The usefulness of the package will be illustrated by analysing data belonging to the HELIX's Spanish cohort where exposome, genome, transcriptome, methylome and proteome data is available joint with information about respiratory and neurocognitive outcomes.

Keywords - exposome, environmental, epidemiology, multiomics, crossomics, software R

SESSION 4: DATA INTEGRATION AND MODELING

MULTIDATASET: AN R PACKAGE FOR ENCAPSULATING MULTIPLE DATA SETS WITH APPLICATION TO OMIC DATA INTEGRATION

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Abstract

Reduction in the cost of genomic assays has generated large amounts of biomedical-related data. As a result, current studies perform multiple experiments in the same subjects. While Bioconductor's methods and classes implemented in different packages manage individual experiments, there is not a standard class to properly manage multiple types of datasets obtained from the same individuals. In addition, most R/Bioconductor packages that have been designed to integrate and visualize biological data often use basic data structures with no clear general methods, such as subsetting or selecting samples.

To cover this need, we have developed MultiDataSet, a new R class based on Bioconductor standards, designed to encapsulate multiple data sets. MultiDataSet deals with the usual difficulties of managing multiple and non-complete data sets while offering a simple and general way of subsetting features and selecting samples. We illustratethe use of MultiDataSet in three common situations: 1) subsetting operations before doing an integration analysis with a third party package; 2) creating new methods and functions for omic data integration; 3) encapsulating new unimplemented data from any biological experiment.

MultiDataSet is a suitable class for coordinate data management and data integration under R and Bioconductor framework.

Keywords - Omics data, Data integration, Data infrastructure, Data organization, R

POSTERS

P01

THE HUMAN EARLY LIFE EXPOSOME (HELIX) PROJECT: MOLECULAR MECHANISMS

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Abstract

The exposome is defined as all the exposures from conception to death. Prenatal and early life exposome is thought to exhibit more damaging effects on health than postnatal insults. The aims of the Human Early Life Exposome (HELIX) project are: i) to characterize the early life exposome, ii) to associate it with child health outcomes, and iii) to relate it to omics imprints and to understand the action molecular mechanisms. The present abstract focuses on the third objective of the project.

The HELIX project consists of 1,300 children of 6-10 years from 6 European cohorts which were followed using the same harmonized protocols. The prenatal and postnatal exposome (>200 exposures) including the outdoor exposome (air pollution, build environment, noise), the individual exposome (cotinine, metals, POPs, PFAS, phthalates, phenols and organophosphates) as well as life style factors was measured. Three main health outcomes were assessed: i) growth and cardio-metabolic phenotype, ii) lung function, asthma and allergy, iii) neurodevelopment and behaviour. In addition, molecular signatures at the age of 6-10y were obtained: blood DNA methylation, gene and miRNA transcription, serum and urinary metabolites and plasma proteins.

The global effect of the exposome on the omics profiles will be analyzed using the O2PLS (orthogonal two partial least squares) regression. This will give us a measure of the variability of the omics profiles that could be explained by the exposome. Linear regressions adjusted for main covariates will be applied to identify molecular biomarkers associated with each exposure. Summarized results will be publicly available.

Three environmental exposures with high concern in public health will be analysed in more detail: tobacco smoking, air pollution and persistent organic pollutants. Exposure to prenatal and postnatal tobacco smoking is a known risk for health outcomes in children. It has been reported that prenatal maternal smoking alters cord blood DNA methylation, with some changes being persistent until adolescence. However, the functional consequences of these changes in terms of gene expression remain unknown and they can be explored in HELIX.

Keywords - HELIX, exposome, omics, prenatal, postnatal

P02

UNTARGETED LIQUID CHROMATOGRAPHY COUPLED WITH HIGH RESOLUTION MASS SPECTROMETRY ANALYSIS OF RICE LIPIDOME UNDER HYDRIC AND HEAT STRESS

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Abstract

Environmental stresses, including heat and hydric stress, are the major factors that limit the geographical distribution of plants. Plants have evolved different strategies to adapt to these climate stresses. From the biochemical viewpoint, maintaining the integrity and fluidity of membranes is critical for plants to survive these climatic stresses. Lipid studies are required to understand this plant adaptation to climate changes. Here untargeted liquid chromatography coupled with mass spectrometry (LC-MS) lipidomics analysis was used to investigate the effects of heat and hydric stress on Japanese rice (Oryza sativa var. Japonica).
Two rice crops were performed, one at normal temperature conditions (range from 22ºC to 28ºC) and the other at high temperature conditions (range from 25ºC to 31Cº). Rice was raised in optimal conditions for 10 days and, after, was watered with four levels of Milli-Q water: 150mL (control), 100mL, 50mL and 5mL. After harvesting, rice roots and aerial parts were separated for subsequent analysis. Chromatographic separation was performed on a Kinetex C18 (100 x 2.1 mm i.d.; 1.7 µm) column. The mass spectrometer was a time-of-flight (TOF) analyzer equipped with an electrospray (ESI) as ionization source working in both negative and positives modes at full scan (50-1500 Da). Finally, multivariate data analysis tools (PCA, PLS-DA, ASCA, and MCR-ALS) were used to analyze the data.
PCA and PLS-DA methods were tested for the classification of rice samples according to temperature and watering treatments. PLS-DA VIP scores plot was used to select those lipids most important for achieving this classification. A total number of 170 lipids were selected for aerial part samples and approximately 200 for root samples. These lipids were tentatively identified using their mass spectra. The majority of the found lipids were accumulated in stressed plants. Glycerolipids (DG and TG) were one of the most altered families. Moreover, at high temperature, the degree of unsaturation of fatty acids decreased. Finally, the biological interpretation of these results was assessed.

Keywords - Environmental stresses, rice, lipidomics, multivariate data analysis.

P03

ASSESSMENT OF ENDOCRINE DISRUPTORS EFFECTS ON ZEBRAFISH EMBRYOS BY UNTARGETED METABOLOMICS

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Abstract

Zebrafish (*Danio rerio*) is a model vertebrate organism for biological, behavioural and biomedical research. In particular, zebrafish embryos are a well-recognized model for environmental risk assessment of chemicals due to their rapid development into larvae (within 48 hours) and their high sensitivity to chemical treatment. In addition, their small size, wide distribution and easy growth conditions offer the possibility to perform small-scale and high-throughput analyses for -omics studies, including metabolic profiling.

In this work, the effects of different endocrine disruptors (EDCs) on the metabolic profiles of five-day-old zebrafish embryos were evaluated using an untargeted metabolomic approach based on hydrophilic interaction liquid chromatography-mass spectrometry (HILIC-LC-MS). Zebrafish embryos were exposed to chemicals commonly released into the aquatic media, such as bisphenol-A (BPA), perfluorooctane sulfonate (PFOS) and tributyltin (TBT).

Collected data were analysed by a combination of different chemometric tools. First, high-resolution MS raw data were compressed using the “region of interest” (ROI) approach to solve storage requirements without loss of spectral accuracy. Multivariate curve resolution alternating least squares (MCR-ALS) was then applied to the simultaneous analysis of multiple full scan HILIC-LC-MS datasets. This strategy permits the resolution of a large number of metabolites being characterized by their chromatographic peaks (elution profiles) and their corresponding mass spectra profiles. After applying ROI-MCR-ALS approach, the significance of the concentration changes of resolved elution profiles (detected metabolites) upon chemicals’ exposure was assessed using several multivariate data analysis approaches. Finally, metabolite concentration changes caused by each EDC were compared. PFOS exhibited less effect than BPA and TBT, which appear to produce a similar subset of altered pathways.

Keywords - Endocrine disruptors, Untargeted metabolomics, Zebrafish embryos.

P04

EXPOSURE TO CHLORPYRIFOS CAUSES MORPHOMETRIC, BIOCHEMICAL AND LIPIDOMIC ALTERATIONS IN GREEN BEANS *(Phaseolus vulgaris)*

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Abstract

Chlorpyrifos (CPF) is an organophosphorate pesticide sold in many countries to control a variety of insects in cotton fields and fruit, nut and vegetable crops. The aim of this study was to evaluate the effects of increasing doses of CPF on phenotypic and oxidation parameters, as well as in the lipidome of green bean plant. Morphological measurements (height, number and size of leaves, root length, number of nodes and pods) were made and several oxidation markers were quantified. The untargeted lipidomic analysis was performed separately in 6 differentiated parts of the plant: roots, stem base, stem, leaves, pods and seeds. The morphological measurements indicated that CPF affects plant growth, being the plant height, the bean length and the number of pods the most decreasing parameters. Also, an increasing tendency of oxidation biomarkers such as lipid peroxidase, glutathione peroxidase and glutathione-S-transferase together with an increase in the protein levels were detected in roots and the stem base, suggesting that CPF triggered the oxidation protective mechanisms of the plant. Concerning the lipidomic analysis, important differences in the content of specific lipid families as well as in the photosynthetic components such as chlorophylls, pheophytin and PQ3 were detected at 6 different levels. The most remarkable changes appeared at the seed level, were a huge reduction on triglyceride (TG) levels was observed. This important change in the lipid composition of seeds of plants treated with CPF evokes two main consequences. On one hand, the decrease of TG content in the seeds would have a clear impact in the reproduction process of the plant, as TGs represent energy storage necessary for seed germination. On the other hand, the important changes observed both in the pods and seeds represent an alteration of nutrients from the point of view of green bean human consumption.

Keywords - Untargeted lipidomics, green bean, chlorpyrifos, oxydation biomarkers

P05

UNTARGETED LIPIDOMIC STUDY OF CHRONIC UV RADIATION EFFECTS IN MELANOCYTES

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Abstract

UV radiation present in sunlight and in artificial light source is an environmental human carcinogen. Epidemiological studies suggest that chronic exposure to UV solar radiation is responsible for skin tumor development via gene mutations and immunosuppression. Solar UV radiation is composed by ultraviolet B radiation (290-320 nm), which is responsible for direct DNA damage, and ultraviolet A radiation (320-400 nm), which causes indirect damage through the generation of reactive oxygen species.

In this work, an untargeted lipidomic analysis has been performed in order to investigate which are the important lipids involved in the effects of UV solar radiation in melanocytes, non-cancerous skin cells. This study was carried out using a solar simulation unit to perform a chronical irradiation at melanocytes.

Experimentally, cells were exposed under chronic conditions, which were optimized using cell viability tests. Then, a lipidomics study was carried out by UHPLC-ToF-MS. Multivariate data analysis (PCA, PLS-DA and MCR-ALS) methods were used to reveal the changes in the concentrations of the main lipids due to UV solar radiation effects in the melanocytes. Once these lipids were identified, they were used to decipher the biologic pathways involved in UV radiation cell damage. Biological proves were carried out to validate these results, such as western blot (WB), qPCR or enzymatic activity assays.

Keywords.- Lipidomics, UV radiation, melanocytes

P06

ROIMCR: A POWERFUL DATA ANALYSIS STRATEGY FOR LC-MS METABOLOMIC DATA SETS

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Abstract

The analysis of LC-MS metabolomic data sets appears as a challenging task in a wide range of disciplines since it demands highly-extensive processing of a vast amount of data (Dettmer, Aronov, & Hammock, 2007). Different LC-MS data analysis packages (e.g., XCMS, MZmine, and MetAlign) have been developed in the last years in an attempt to facilitate this analysis. However, most of these strategies involve chromatographic alignment and peak shaping and often associate each "feature" (i.e., chromatographic peak) to a unique m/z measurement, an assumption that is not necessarily true (distinct m/z values can describe the same elution profile).
Here we present an alternative chemometric approach called ROIMCR to: i) compress massive LC-MS data while transforming their original structure into a data matrix of reduced dimensions without missing relevant information through the search of regions of interest (ROI) in the m/z domain (Gorrochategui, Jaumot, Lacorte, & Tauler, 2016; Tautenhahn, Bottcher, & Neumann, 2008) and ii) resolve compressed data to find their contributing pure components without previous alignment nor peak shaping by applying Multivariate Curve Resolution-Alternating Least Squares (MCR-ALS) analysis (Jaumot, Gargallo, de Juan, & Tauler, 2005; Tauler, 1995).
The applications of the LC-MS untargeted data analysis strategy are shown for a human placental choriocarcinoma cell line (JEG-3), used as a toxicological model to simulate the effects of tributyltin (TBT). Overall, the hereby presented ROIMCR strategy demonstrates the usefulness of chemometrics in LC-MS data analysis and it is a valuable addition to the untargeted metabolomic research.

Keywords - Data analysis, Chemometrics, Omics, Untargeted, LC-MS

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P07

UNTARGETED METABOLOMIC PROCESSING OF BLOOD SAMPLES FROM PRETERM INFANTS STORED WITH RNA STABILIZATION REAGENT

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Abstract

In transcriptomics studies, the use of a stabilizing reagent that inactivates cellular RNAses and selectively precipitates RNA while maintaining genomic DNA and proteins in solution is necessary to preserve the bood sample during storage. For carrying out integrative omics approaches in preterm infants, where access to blood sample volumes is limited, the sample collection protocol should ideally be compatible with all omics techniques. However, the generally employed stabilizing reagent (i.e. chaotropic agents and detergents) makes the sample incompatible with Gas and Liquid Chromatography coupled to Mass Spectrometric detection (GC-MS and LC-MS). Herewith, we have developed a complete pipeline for untargeted GC-MS and LC-MS metabolomics of whole blood samples stored in Tempus® tubes. 20 samples from newborns were collected in Tempus® tubes and stored at -80 ^{0}C until processing. Precipitation with different amounts of methanol and acetonitrile prior to GC-MS and LC-MS analysis respectively were optimized. The results show that this workflow allows to simultaneously obtain transcriptomics and untargeted metabolomics signatures from the same blood sample. Detected metabolic features were putatively annotated demonstrating the practical usefulness and metabolome coverage. Future studies will apply this workflow for studying the effect of sepsis on the metabolome of preterm infants.

Keywords - Stabilizing reagent, LC-MS, GS-MS, untargeted metabolomics, neonatology.

P08

ANALYSIS OF NEUROBEHAVIOURAL DATA BY CHEMOMETRIC METHODS IN ECOTOXICOLOGICAL STUDIES

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Abstract

Incorporation of chemometric tools in behavioural data management workflows allows for the early identification of most relevant endpoints complementarily to statistical confirmatory approaches. In this work, the effects of two model neurotoxicants, chlorpyrifos (CPF) and nicotine, exposures on behavioural profiles of adult zebrafish at three different times (2, 6 and 24 h) were evaluated using open field test (OFT) paradigm experiments. Two chemometric methods like Principal Component Analysis (PCA) and Analysis of Variance-Simultaneous Component Analysis (ASCA) have been used to interpret the changes observed in the obtained behavioural data. A decreased of the locomotor activity, an anxiolytic effect and an altered exploratory behaviour were the most affected behavioural endpoints in the CPF exposures. However, an increase of the locomotor activity and an anxiogenic effect were observed in the nicotine exposures. Finally, an excellent correlation between the ASCA results and the results obtained using traditional statistical procedures for both compounds were encountered.

P09

THE HIDE-AND-SEEK CHALLENGE OF 1H-13C HSQC NMR METABOLOMICS

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Abstract

Metabolomics is a field of 'omics' research focused on the characterization of small molecule metabolites in cells, tissues, organs and organisms[1]. Metabolomics is one of the most ambitious 'hot topic' at present, since it is being used for biomarker discovery[2], environmental assessment[3], and phenotyping[4], among others.
One of the preferred instrumental techniques in metabolomics is Nuclear Magnetic Resonance (NMR) spectroscopy. From those studies using NMR, most have been focused on the analysis of one-dimensional proton (1D 1H) NMR, while the analysis of other nuclei (such as 13C and 31P) and other NMR experiments (1H-13C HSQC[5], 2D 1H INADEQUATE[6],…) are still underrepresented. The preference of 1D 1H NMR in NMR metabolomics lies in the fact that has good sensitivity in a short acquisition time, albeit it lacks spectral resolution since it presents a high overlapping degree.
In this study, we have characterized the metabolome of a yeast strain grown in two different liquid media with 1D 1H NMR and 2D 1H-13C HSQC experiments, giving a total of 64 unique measured samples containing hundreds of resonances each.
After careful scrutiny of the data, dozens of metabolites, including amino acids, nucleotides, sugars and organic acids, among others, have been detected and quantified. Some of the resonance assignments could not be performed using only the 1D 1H NMR spectra, as they were hidden under other (major) resonances in the proton dimension. In addition, in the 1H-13C HSQC spectra, some unexpected long-range JCH couplings signals were camouflaged among the 1JCH resonances, which were used for a more reliable metabolite characterization.
Even though the acquisition time of a 1H-13C HSQC experiment is longer than for 1D 1H NMR experiments, with this empirical study we have concluded that its acquisition and its further analysis is desirable, since a better coverage of the overall metabolome can be obtained.

Keywords - HSQC, yeast, NMR

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P10

MUSCLE LIPIDS OF FISH MAY BE AFFECTED BY ENVIRONMENTAL POLLUTION?

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Abstract

Mediterranean Rivers are strongly affected by pollution and water scarcity. Specifically, over the summer period, urban and industrial discharges arrive into the river with little dilution and, consequently, fish inhabiting these rivers are highly exposed to anthropogenic chemicals. Recent studies have evidenced that contaminants, including mammalian obesogens, are able to disrupt lipid homeostasis in fish. However, scarce information is available on lipid alterations of field exposed organisms. This work aims at analysing muscle tissue lipidome of an autochthonous fish species, and the potential alterations along a pollution gradient. Thus, Barbus meridionalis were collected from six sites along the Ripoll River and lipids were extracted from a subsample of the muscle tissue, and analysed by ultrahigh resolution mass spectrometry coupled to high resolution mass spectrometry with a time of flight analyzer (UHPLC-ToF). Lipid analysis allowed the identification and quantification of about 130 lipids in muscle tissue, including phosphatidylcholines (PC), PC-plasmalogens (PC-Ps), cholesterol esters (CEs), triacylglicerols (TGs), diacylglicerols (DGs) and sphingomielins (SMs). Principal component analysis (PCA) were applied to compare the muscle lipid profiles of fish from different sampling sites, allowing a clear separation of the lipidome of fish from polluted and reference sites. Specifically, a decrease of the most polyunsaturated PCs was found together with an accumulation of CEs, TGs, DGs and saturated SMs in fish from polluted areas, whereas a concomitant enrichment of PC with 5-7 double bonds was observed in muscles of fish from the reference sites. Exposure to xenobiotics can enhance the production of reactive oxygen species (ROS), which if not detoxified, may interact with cellular macromolecules causing oxidative tissue damage and lipid peroxidation, being those PCs containing polyunsaturated fatty acids the most susceptible to oxidation. The study evidences significant changes in muscle tissue lipidome of Barbus meridionalis in the lower course of the Ripoll River as a consequence of exposure to pollutants from urban and industrial origin.

Keywords - Fish, Mediterranean rivers, pollution, lipidomics, LC-MS.

P11

GPCA FOR IMPROVED MULTIVARIATE ANALYSIS INTERPRETATION IN LIPIDOMICS

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Abstract

Multivariate Analysis (MA) has emerged as a powerful tool for supporting lipidomic investigations [1]. Individual lipid species, lipid families, or specific lipid changes from sample to sample can be easily revealed using multivariate statistical procedures. To this end, both unsupervised (e.g., principal component analysis: PCA) and supervised (e.g., partial least-squares: PLS) algorithms can be used [2]. However, the tendency of lipidomic tools is to make multivariate statistical analysis as simple as possible for the user, leading in many cases to “black boxes” in which advanced data interpretation is very limited. Furthermore, interpretation of standard MA tools, like PCA loading plots, may be challenging due to the dimensionality of the data, since the principal components are linear combinations of all the variables simultaneously.
To overcome these limitations, here we demonstrate that Group-wise Principal Component Analysis (GPCA) [3], a recently proposed extension of PCA for exploratory analysis, can be successfully applied as a user-friendly advanced tool for the visualization and interpretation of the statistical analysis. GPCA starts from the groups of variables identified by MEDA (Missing-data for Exploratory Data Analysis) [4] and performs a constrained PCA-like calibration where loadings are restricted to present non-zero values only for a group of variables. In this way, the obtained Group-wise PCs (GPCs) are sparse factorizations that can be inspected individually (one GPC at a time), simplifying interpretation. To further make MA outcomes interpretation easier and faster, we also introduce here the Discriminative score (D-score), which assesses the discriminative power of each GPC according to an arbitrary desired clusterization, in turn allowing the ranking of the GPCs.
Examples of how and why GPCA combined with the D-score are valuable solutions for the exploration and interpretation of complex real lipidomic case studies will be given.

Keywords - Lipidomics, Multivariate Analysis, GPCA

Acknowledgement

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P12

MERCURY EFFECTS IN SALT MARSH PLANTS: ALTERATIONS IN THE METABOLOME OF HALIMIONE PORTULACOIDES FROM A HIGHLY CONTAMINATED SYSTEM

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Abstract

Salt marshes are one of the most productive ecosystems. They serve as sink for contaminants, namely metals, from industries and municipal effluents. A confined basin (Laranjo) from a Portuguese costal system (Ria de Aveiro) received for several decades an effluent rich in mercury (Hg), increasing the levels of Hg in this basin with unpredictable consequences for the life it supports.
With the objective to unveil the influence of Hg contamination on the salt marsh plant community, Halimione portulacoides plants, a halophyte frequent in the Ria de Aveiro, was collected from two sites in Laranjo basin differing in Hg concentration. Hg concentration was determined in the sediment and different plant organs and the bioaccumulation and translocation factors were calculated. Biochemical alterations, including the antioxidant response, were determined. The alterations induced by Hg to the lipidomic profiles were analysed. Results showed differences in accumulation and distribution of Hg in the plant. The highest concentration of Hg was found in roots, but restriction of Hg translocation lost efficiency at the most contaminated site. The biochemical analysis evidenced differences between organs and environmental Hg contamination, with organs from the most contaminated site displaying higher levels of oxidative stress and cell damage. Lipid profile also show differences between plant organs of the two sites; Hg reduced galactolipids and chlorophyll contents, reflecting the higher oxidative stress; α-tocopherol increased providing protection from peroxidation to thylakoid membranes, and reducing H influence on photosynthetic activity; phospholipids also decreased, reducing the instability in lipid bilayers and making membranes less permeable to ions such as Hg. These results suggest that Hg induced important changes in the composition salt marsh plants lipids and can compromise crucial plant processes.

Keywords - Salt marshes, mercury, oxidative stress, UPLC-MS, lipidomic, biochemical response, bioaccumulation

P13

METABOLIC FLUX ANALYSIS OF GLUCOSE-LACTATE CONCOMITANT CONSUMPTION IN CHO CELL CULTURES

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Abstract

Conventional metabolism of CHO cells is characterized by the consumption of large quantities of glucose and concomitant production of large quantities of lactate, a by-product widely reported to inhibit cell growth in culture. Recently, we have observed that under certain culture conditions, CHO cells are able to co-metabolize glucose and lactate, even during the exponential growth phase.
In our lab, three different metabolic behaviors have been observed in bioreactor depending on the culture conditions. In pH controlled cultures (set at 7.2) glucose was consumed and lactate was produced at high rates (phase 1). Once glucose was completely depleted, lactate was consumed as a sole carbon source, but under such conditions no cell growth was observed (phase 3). In non-controlled pH cultures, phase 1 was also reproduced until pH dropped below 6.8 due to lactate accumulation in the broth. At this point, glucose and lactate concomitant consumption was triggered (phase 2) without significant effect on cell growth rate.
These different metabolisms have been characterized for the first time in CHO cells by means of Flux Balance Analysis (FBA) using a reconstruction of genome-scale metabolic model. FBA confirmed that in phase 1, pyruvate generated through glycolysis is converted to lactate to fulfill the NADH regeneration requirements in the cytoplasm and only a small amount of pyruvate (29%) is introduced into TCA through Acetyl-CoA. Differently, in glucose-lactate concomitant consumption (phase 2), glucose uptake is significantly reduced up to 4-5 folds, observing a balance between glycolysis and TCA cycle. Such novel metabolic behavior yields to a more efficient substrate consumption without secreting non-completely oxidized by-products. Moreover, two different models considering alternative cytoplasm or mitochondrial lactate dehydrogenase metabolic phase 2 were discussed, due to the controversy between different authors in how lactate reaches mitochondria and in which form is transported into the mitochondrial matrix.

Keywords - Cell Culture, CHO, Flux Balance Analysis, Genome-scale metabolic model, Glucose/Lactate Concomitant Consumption, Lactate, Metabolic shift, Warburg effect

P14

BACTERIAL VOLATILES AS PLANT GROWTH PROMOTERS – DETECTION BY THE COMBINED APPLICATION OF HS-SPME, GC-MS AND MCR-ALS ANALYSIS

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Abstract

Some soil bacteria provide benefits to plant productivity such as nitrogen fixation, improved assimilation of nutrients and production of phytohormones. In recent years, a novel mechanism of growth promotion has gathered significant attention from researchers interested in soil biology and agricultural sciences.
Early reports show that volatile organic compounds (VOCs) produced by bacteria can promote plant growth, induce resistance to diseases, improve tolerance to abiotic factors such as drought and salinity, enhance nutrient acquisition by plants, act as antagonistic agents against phytopathogenic organisms or increase photosynthetic rates. Due to their volatile nature, these compounds can be extracted without the use of solvents by headspace solid-phase microextraction (HS-SPME) and there is also no need for derivatization, since they can be analysed directly by gas chromatography-mass spectrometry (GC-MS). However, the detection of these compounds is limited by co-elution problems and by the generation of complex data sets from the high-throughput platforms, which together make the data analysis a challenging bottleneck. Here we report growth promoting effects in Arabidopsis thaliana induced by rhizobacterial volatiles. These effects were observed in bioassays in which centre divided plates were used. The centre partition denied physical contact between the plants and the bacteria. However, the atmosphere was shared and therefore the growth promoting compounds had a volatile nature. Since the bacteria were isolated from the nodules of legumes and the model used in this study was a non-legume, this might be a non-specific mechanism of growth promotion. We also report the analysis of the VOCs emitted by several rhizobacterial strains that promoted growth of A. thaliana by HS-SPME and GC-MS, and the application of the chemometric method multivariate curve resolution-alternating least squares (MCR-ALS) to the data processing to improve the detection of VOCs that can be co-eluted.

Keywords - Plant growth promoting bacteria, Gas chromatography-mass spectrometry, Multivariate curve resolution-alternating least squares

P15

IDENTIFYING BIOMARKERS BY INTEGRATING MULTIPLE-OMICS DATASETS TO IMPROVE ANAEROBIC DIGESTION

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Abstract

Anaerobic digestion (AD) is a complex microbiological process of degradation of the organic matter which produces biogas rich in methane. It is used to manage different types of waste and produce energy at industrial scale. However, AD is not fully controlled and exploited, mainly because of the absence of microbial-based management tools. Omics methodologies now open up the possibility to deepen the knowledge of AD microbiota and to develop biomarkers for digester functioning diagnosis.
In this framework, the influence of different parameters on the stability of anaerobic digester (inhibitors, micropollutants, temperature, etc.) was tested at lab-scale. Circa 120 samples, corresponding to various conditions of functioning, were taken and analysed with omics. Four datasets were produced. Degradation performances were monitored through several physicochemical indicators (gas production kinetics, dissolved carbon, etc.) (1). Microbial dynamics and activity were analysed through 16S sequencing of both DNA (2) and RNA (3). Metabolic pathways were investigated with non-targeted metabolomic analyses (high-resolution mass spectrometer-LTQ-Orbitrap XL) (4).
Classical multivariate analyses (PCA, NMDS, PLSDA) were applied to the different datasets separately to set-up a first classification of the samples. A new analytical framework of mixOmics R package, mixDIABLO, was then used for the integration of the different data sets in a supervised analysis, according to the different conditions of functioning. It enabled to extract complementary information from the data, to gain a better understanding of the interplay between microbial dynamics and activity, metabolic pathways and macroscopic degradation performances. In parallel, sparse Generalised Canonical Correlation Analysis was used to identify correlated variables in the different datasets, in order to explain good and bad performances observed. Specific microorganisms and molecules, characteristic of the different conditions of functioning were identified and we believe that they could be used as biomarkers for functional diagnosis of AD digesters.

Keywords - Keywords - complex microbial community – metabolomics – 16S

P16

COMBINED USE OF CHEMOMETRICS AND HYPERSPECTRAL IMAGES FOR ENVIROMENTAL –OMICS STUDIES APPLIED TO RICE PLANTS

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Abstract

Metabolomic changes in biological plants tissue could be generated due to exposure to environmental contaminants. The aim of this research is to investigate the effect of heavy metals on rice plants using hyperspectral Imaging combined with chemometrics tools that allows providing simultaneously spatial and spectral information about the sample.
Japanese Rice (Oryza sativa var. Japonica) was exposed to different concentrations of heavy metals Cu(II) and Cd(II) (50 μM and 1000 μM). After harvesting, rice leaves and stems were cut in small sections, embedded in OCT polymer and flash frozen in liquid N2. 18 μm thick cryosections were obtained and imaged by Raman and fluorescence spectroscopy.
Multivariate Curve Resolution Alternating Least Squares (MCR-ALS) was the chemometric method mainly used to interpret the large number of information obtained by hyperspectral images. MCR-ALS provides pure spectra profiles and distribution maps for each biological components of the sample. Multiset analysis has been performed to analyze separately images acquired on each of the different populations (Control, exposed to low and high level of different heavy metals) in order to interpret better the effect of heavy metals on rice plants.
Besides, in plant tissues, the combination between Raman and autofluorescence spectral imaging is helpful to clarify the difference between each biological component of the sample and to have a global description of the plant tissue. This fact is linked to the similar spatial resolution of both imaging techniques and to the complementary information they offer. On the one hand, Raman spectroscopy is a very rich structural technique helpful to identify certain plant constituents, but unable to scan vegetal tissues with high autofluorescence; on the other hand, highly autofluorescent tissues have natural fluorophores that can be easily monitored and yield information when scanned by fluorescence imaging techniques.

Keywords - Heavy metals in plants, Hyperspectral Imaging, MCR-ALS, Multiset Analysis

P17

ASSESSMENT OF THE EFFECT OF TIME EXPOSURE OF CHLORPYRIFOS-OXON ON DIFFERENT PHENOTYPES OF ZEBRAFISH EMBRYOS BY HYPERSPECTRAL IMAGING AND CHEMOMETRIC METHODS

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Abstract

Hyperspectral imaging techniques (HSI) provide spatial and chemical information and preserve the natural morphology of the samples. These properties make HSI appropriate for biological and biomedical studies, and its use has increased in recent years. However, hyperspectral images precise the use of chemometric techniques to handle the high amount of information they provide. In this study Multivariate Curve Resolution-Alternating Least Squares (MCR-ALS) is used to obtain the pure spectral signatures and distribution maps of compounds in an image or multiset structure that contains several images.
The aim of this work is the assessment of the effect of time exposure of chlorpyrifos-oxon (CPO) on different phenotypes (P2 and P3) of zebrafish embryos by hyperspectral imaging. To do so, a group of zebrafish embryos have been exposed to CPO (concentration of 3μm) and some specimens with the P2 and P3 features have been collected at 6 and 24 h of exposure and imaged using a Raman microscope. A multiset structure has been built for each combination of phenotype and time of exposure and analyzed by MCR-ALS.
At this point, a qualitative interpretation of the effect of time exposure of the contaminant to each phenotype can be studied. However, in order to assess and model time exposure to CPO and the phenotype effects, a new approach to use ANOVA-Simultaneous Component Analysis (ASCA) on HSI data is performed. This approach consists of a separate resampling strategy for each of the multisets analyzed. For each specific multiset many small multisets are derived that contain representative pixels of all images. Each small multiset has been analyzed by MCR-ALS. The spectral signatures for a particular component in the four resampled multisets are arranged together and submitted to ASCA. In this way, an ASCA model is built for each resolved component. ASCA results are used to assess and model the time exposure of CPO and phenotype effects.

Keywords - Hyperspectral imaging, ANOVA-Simultaneous Component Analysis (ASCA), Multivariate Curve Resolution- Alternating Least Squares (MCR-ALS), Raman spectroscopy, environmental -omics

P18

MODELLING THE DURATION OF HYPOXIA: A PLASMA METABOLITE SCORE

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Abstract

In term and near-term babies, hypoxic-ischemic encephalopathy (HIE) secondary to perinatal asphyxia is a leading cause of mortality and long-term neurologic co-morbidities. The most successful intervention for treatment of moderate to severe HIE is moderate whole body hypothermia initiated within 6 hours from birth. This work strives for an early metabolic biomarker score with the aim of providing an objective measure for assessing the risk of developing moderate to severe HIE, thereby supporting clinical decisions. From a consolidated piglet model for neonatal hypoxia-ischemia, plasma samples were collected before and at different time points after a hypoxic insult. A set of three metabolites (choline, xanthine and hypoxanthine) showing maximum correlation with hypoxia time was identified from an untargeted liquid chromatography coupled to tandem mass spectrometry experiment. A metabolite score based on Partial Least Squares regression was used for predicting hypoxia time. Its performance as a biomarker for perinatal asphyxia was compared to lactate, which is currently considered as the gold standard. The metabolite score performed similar to lactate for plasma samples withdrawn before and directly after hypoxia, both providing sensitivity and specificity values equal to 1. However, for samples collected 2 h after resuscitation, lactate levels were not suitable for identifying asphyxiated piglets, while the metabolite score improved the predictive capacity. Results evidenced the usefulness of the metabolite score for an early assessment of the severity of hypoxic insults based on serial determinations in plasma samples. Ongoing studies are testing its capacity for diagnosis and patient stratification in multicenter clinical trials involving newborns with HIE.

Keywords - LC-MS metabolomics, birth asphyxia, piglet model.

P19

EVALUATION OF BATCH EFFECT ELIMINATION USING QUALITY CONTROL REPLICATES IN LC-MS METABOLITE PROFILING

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Abstract

Systematic variation of the instrument's response is a frequently observed issue in untarget LC-MS metabolomics implying the analysis of a large number of biological samples. The so-called batch effect decreases the statistical power and has a negative impact on repeatability and reproducibility of the results. This work explores the effectiveness of a set of qualitative and quantitative tools for batch effect assessment based on the use of quality control (QC) samples by means of an LC-MS dataset obtained during the analysis of over 316 urine samples and 76 QCs in 3 different analytical batches. Qualitative tools include the monitoring of spiked internal standards, principal component analysis (PCA) and hierarchical cluster analysis (HCA). Quantitative tools comprise the distribution of RSDQC values and the mean Pearson correlation coefficient in QCs, the ratio of random features in QCs using the runs test, as well as the δ-statistic, Silhouette plots, Principal Variance Component Analysis (PVCA) and the expected technical variation in the prediction. Two algorithms for batch effect removal based on modeling the signals obtained during the measurement of QC samples are used. This work shows that the graphical integration of outputs from multiple data quality assessment tools offers a straightforward way for tailoring batch effect elimination approaches and facilitates the comparison across data pretreatments.

Keywords - Metabolomics, batch effect, experimental design, QC-SVRC, QC-RSC

P20

OUTFITTING THE FACTORY OF THE FUTURE WITH ON-LINE ANALYSIS

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Abstract

Chemometrics integrates domain knowledge of the samples, knowledge about the analytical platform and multivariate analysis. The 'Outfitting the Factory of the Future with ON-line analysis' (OFF/ON) project aims to use this integrated approach in the comprehensive modelling of (industrial) processes. Currently used multivariate data analysis methods from Process Analytical Technology (PAT) are very well able to detect errors in a specific process step, but there is a considerable gap in the available methodology for integration of measurements from different process locations in combination with data of different levels of quality and precision. The challenges encountered here are highly analogous to those in the omics field, where the data is inherently of poor quality, highly variable and may originate from different sources within the same subject.
We aim to provide innovative and generic chemometric and statistical methods for process monitoring using all available data, drawing inspiration from the omics field. The processes we study range from chemical plants to food safety and surface water quality. For example, we are working on a multivariate water quality index, combining measurements from multiple locations along the river.
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Keywords - Chemometrics, process analytical technology, water quality index

P21

BISPHENOL A (BPA) AS A DISRUPTER OF LIPID METABOLISM IN ZEBRAFISH EMBRYOS

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Abstract

Bisphenol A (BPA) is a plasticizer known as a xenoestrogen and a potential obesogen. Although its use has been regulated around the world, there are still large uncertainties on its effects at embryonic stages, both at short and long term.
In order to identify molecular footprints of the exposure to BPA, we exposed zebrafish embryos during 2-5 dpf (days post fertilization) to different BPA concentrations (control, 0.1, 1 and 4 mg/L) to analyze effects at transcriptomic, metabolic and morphological levels at these early embryonic stages.
Transcriptome changes were analyzed by RNA seq (5dpf, 3 replicate/condition, 10 embryos/replicate). Statistical analysis using DESeq2 (R package) revealed 1517 differentially expressed genes (DEGs, adjusted p-value <0.05).
Functional analyses of DEGs revealed a significant enrichment in genes related to lipid metabolism. To explore this effect, we analyzed total lipid profiles of control and exposed embryos (4, 6 and 8 mg/L of BPA) at 4, 5 and 6 dpf, by high-performance thin layer chromatography (HPTLC). This technique provides an integrated system to measure the different lipid classes and its relatively high throughput facilitates dose-response studies under very different parameters. The results showed not only that BPA-treated animals maintain a significant proportion of triglycerides (TAGs) until at least 6 dpf, but also changes in most other lipid classes during the 4-6 dpf period.
These results agree not only with the transcriptomic data, but also with morphological analyses showing a significant tendency to retain yolk sac remnants after 5 dpf, and an increase in yolk sack size in BPA-treated embryos at early stages. This demonstrates the physiological connection between gene deregulation, altered metabolic profiles, and morphological alterations.
We propose that this effect reflects either a yolk sac malabsorption syndrome or an impairment of the general lipid metabolism, which in turn would slower yolk sack consumption.

Keywords - Transcriptomics, lipids, thin layer chromatography, zebrafish, embryo, larvae, BPA, Bisphenol A, endocrine disruption, EDCs

P22

ALTERED LIPID METABOLISM IN DAPHNIA MAGNA EXPOSED TO ECDYSTEROIDS, JUVENOIDS AND BISPHENOL A

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Abstract

The analysis of lipid disruptive effects in invertebrates is limited by our poor knowledge of the lipid metabolic pathways and of their complete lipidoma. Recent studies showed that tributyltin and juvenoids activated the ecdysteroid, juvenile hormone and retinoic X receptor signalling pathways, and disrupted the dynamics of lipid droplets in the crustacean Daphnia magna, promoting their accumulation in post-spawning females. Adult daphnia accumulates triacylglycerols during the reproductive/molting cycle and allocates part of those lipids to eggs. Tributyltin increased lipid droplets in adults was related to a reduced transfer of triacylglycerids to eggs, promoting their accumulation in adults. The present study aims to study how juvenoids, ecdysteroids and bisphenol A disrupt the dynamics of phospholipids and neutral glycerolipids lipids in adult daphnias during the reproductive cycle. Using the web database, LipidMaps (http://www.lipidmaps.org/), a complete database of all the biological known lipids was created, from which 235 lipids were detected by UHPLC-HRMS (TOF) in D. magna samples, compared to the 116 detected in previous studies. The identification of all lipid classes was based on exact mass characterization with an error < 5 ppm, retention time and correct isotopic distribution. Obtained results were validated using HPTLC (high performance thin layer chromatography), being both quantitative and qualitative results well correlated. This study proposes an innovative integration of quantitative and qualitative lipidomic analysis, which allowed identifying new lipids previously unknown in D. magna improving the detection of changes in Daphnia's lipid profile.

Keywords - Obesogen, Lipid disruptor, lipidomic, Daphnia magna

P23

DIBUTYL- AND BENZYLBUTYL-PHTHALATE INDUCE THE ACCUMULATION OF TRIACYLGLYCEROLS IN HUMAN PLACENTAL JEG-3 CELLS

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Abstract

Phthalates are chemicals used as plasticizers to improve the durability and flexibility of plastic products. They can migrate into food and liquids and humans are continuously exposed to these compounds through ingestion. They are suspected to disrupt key pathways involved in lipid metabolism, which is of particular concern in placental cells, due to its involvement in the transport of lipids to the fetus compartment. The aim of this study was to assess the ability of two phthalates widely used as plasticizers, dibutyl phthalate (DBP) and benzyl butyl phthalate (BBP), to alter the lipid composition in human placental JEG-3 cells. Cells were exposed for 24 h to DBP and BBP (5, 10 and 20 μM). These concentrations were not cytotoxic for placental cells, apart from the highest one which lead to 20 % cytotoxicity after 24 h of exposure. Lipid composition was analysed by flow injection analysis coupled to high resolution mass spectrometry (FIA-Orbitrap-Exactive). The analysis allowed the detection of 130 lipid species, including 36 triacylglycerols (TGs), 15 diacylglycerols (DGs), 22 phosphatidylcholines (PCs), 20 PC-plasmalogens (PC-Ps), 20 phosphatidylethanolamines (PEs), 9 PE-plasmalogens (PE-Ps), 8 phosphatidylserines (PSs). In order to detect significant changes in lipid composition of exposed cells, a Partial Least Squares-Discriminant Analysis (PLS-DA) was performed. It showed that exposure to DBP and BBP had similar effects, triggering an accumulation (up to 2.5 fold) of polyunsaturated TGs (52:5, 52:6, 54:5 and 54:6), whereas a depletion (up to 73 %) of several DGs (e.g. 32:1, 34:1, 34:2, 36:2 and 36:3), PCs (e.g. 34:1, 36:2 and 38:2) and PC-Ps (e.g. 36:1, 36:2, 38:3, 38:4 and 38:5), among others, was observed. The results indicate significant changes in JEG-3 cell lipidome after exposure to environmentally realistic concentrations of DBP and BBP. These changes consisted in a significant accumulation of TGs with a concomitant decrease in some membrane lipids, particularly PC-Ps and some PCs.

Keywords - Phthalates, lipids, human placental cells JEG-3

P24

A COMBINED MULTI-OMICS AND IN SILICO APPROACH TO DECIPHER METABOLIC NETWORK SHIFTS DURING THE DIFFERENTIATION OF HUMAN HEPATOCYTE

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Abstract

The hepatic cell line HepaRG, which is increasingly used in toxicity studies, has the particularity to differentiate from progenitor to mature hepatocyte-like cells. In order to explore the metabolic shifts occurring during this differentiation process, we identified the functional metabolic network of HepaRG cells at each developmental stages: day 3 (progenitors) and day 30 (differentiated cells). Gene expression and metabolomic (1H NMR) data obtained from HepaRG cells at the two stages were integrated in the context of the global human genome-scale metabolic network Recon2. We used a modified version of the iMAT algorithm developed by Shlomi et al. to identify, based on these data, the sub-networks of reactions specifically active in HepaRG cells at each developmental stage. For each stage, we identified several sub-networks of active reactions, having an equivalent adequacy to experimental data. We applied classification analysis methods to explore intra- and inter-stages variability among these sub-networks. We showed that, for each stage, the heterogeneity between sub-networks was mainly caused by the occurrence of several alternative reactions or the relative low contribution of transcriptomic data in some pathways. To better characterize the systemic metabolic capacities of the cells, we chose, contrary to most approaches, to consider the whole set of similarly adequate sub-networks, since it allows taking into account various metabolic alternatives. Through simulations and pathway enrichment analyses, we predicted that differentiated cells would globally be able to perform a larger number of liver-specific functions (e.g., urea production) and we identified several sets of reactions that were differently active between the two stages. These reactions mostly belong to pathways specific to hepatic activity (e.g., bile acid synthesis) but also to fatty acid synthesis and oxidation pathways. About 50% of the predicted modulated reactions were not evidenced from transcriptomic data and were « newly » inferred by the computational models.

Keywords - Metabolic network modeling, Transcriptomic, Metabolomic, Hepatic cell line

P25

IDENTIFICATION OF BIOACCUMULATIVE XENOBIOTICS IN MUSSEL FOLLOWING A METABOLOMIC APPROACH: HUMAN AND ENVIRONMENTAL EXPOSURE (XENOMETABOLOMIC PROJECT)

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Abstract

Chemical contamination of estuarine and coastal areas is a highly complex issue with negative implications for the aquatic environment, human health (trough the possible ingestion of contaminated seafood), and related coastal activities such as fishing, aquaculture, or recreational activities. Thousands of chemicals are released to the environment due to human activities generating a “cocktail” of hazardous substances. Given that it is unrealistic to assess every possible combination of chemical substances the major challenge now is to develop systematic ways of addressing these chemical mixtures in environmental assessment, and to identify priority mixtures of potential concern. The application of non-target analysis techniques seems to be the way forward in order to fill this knowledge gap. In this sense, the XENOMETABOLOMIC project presented here offers a new tool based on the application of xenometabolomics to wild organisms that will complement traditional environmental risk assessment techniques. The profiling of the xenometabolome will allow establishing priority mixtures of contaminants, and the metabolome will detect early stage metabolic dysregulations providing relevant information in risk assessment of environmental toxicant mixtures. Besides, the bioaccumulation of these priority mixtures in organisms such as mussels, highly consumed by the population, highlight the necessity of studying not only the identity but also the amount of contaminants present and the effect of cooking on their levels. These issues are tackled in the project with a multidisciplinary approach that will allow first to identity priority mixtures of contaminants of potential concern, second to study their bioaccumulation, third to assess their environmental risk, and fourth to know the real levels of contaminants to which consumers are exposed after cooking a meal with mussel.

Keywords - Xenometabolome, metabolome, priority contaminants mixtures, mussel, bioaccumulation, biomarkers, cooking, risk

P26

HYBRID TARGET/NON-TARGET PROFILING OF FOOD AND ENVIRONMENTAL SAMPLES

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Abstract

As the “omics” world moves towards exposomics studies which consider the exogenous substances to which an organism is exposed, general purpose tools are needed in order to characterize the environment to which an organism is exposed. This includes tools to measure known target molecules of concern, along with unknown/unexpected compounds which could be found in a sample. CAS Registry database contains more than 130 million organic and inorganic chemicals and more than 15,000 new chemicals are being added each day. Many of the small organic compounds that make up the “exposome” are small molecules which are amenable to GC analysis (e.g.: petroleum-based compounds, flavours, fragrances, pesticides, PCBs, PCDD/Fs, etc.). As a platform, comprehensive two-dimensional gas chromatography (GC×GC) with a time-of-flight mass spectrometer (TOFMS) is a nearly ideal tool for studies of an organism’s exposure to a variety of compounds. It offers improved sensitivity over one-dimensional methods, improved separation capacity, leading to cleaner spectra of compounds for identification, minimal matrix effects (when compared with LC-MS), and a broad dynamic range. However, the greatest advantage is that the technique “sees all” with the MS by capturing full mass spectra at every point in the chromatogram. Thus it opens the door to hybrid target/non-target techniques. Presented here is such a method aimed at performing measurements of a host of compounds in aqueous samples (water, beverages) and in solid samples (foods). Semi-quantitative screening methods relying on headspace solid-phase microectraction will also be demonstrated. Additionally, the instrument configuration for this work and for screening biological samples (urine, plasma, feces, etc) is identical, allowing for the easy measurement of what is going on inside and outside of an organism.

Keywords - GC×GC-TOFMS, Exposomics, Environmental analysis, Food, Water, Screening

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TOWARDS AN ATLAS OF THE ZEBRAFISH METABOLOME BY 1H-NMR

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Abstract

Despite its long tradition in metabolomic studies, 1H-NMR has been scarcely used for quantitative analyses of complex samples. In a previous work, we developed an analytical pipeline to evaluate the metabolome of the yeast Saccharomyces cerevisiae by 1H-NMR, which allowed the identification of subtle changes in the yeast metabolism induced by both exogenous and endogenous factors. Now we attempt to apply a similar approach to characterize the zebrafish metabolome, starting by evaluating quantitative and qualitative differences between adult tissues (brain, muscle) and embryos. We detected more different putative compounds in fish than in yeast, according to the former's greater metabolic complexity, and we observed significant differences among tissues, also in agreement with the expected metabolite heterogeneity. An extensive assignment of the about 500 proton resonances observed in the three tissues was achieved by targeted metabolite profiling after consulting both open access (i.e. HMDB) and in-house NMR databases, and by structural elucidation after acquisition of different 2D NMR sequences. In total, we identified about 40 compounds, including amino acids, nucleotides, neurotransmitters, and metabolites from the glycolysis pathway and the Kreb's cycle. We intend to apply the multivariate data analysis approach developed for yeast to identify metabolome alterations induced by toxicants (neurotoxins, endocrine disruptors, teratogens) in zebrafish at different life stages.

Keywords - Toxicology, Danio rerio, chemometrics, omics, nuclear magnetic resonance

P28

MULTIGENERATIONAL EXPOSURE TO CUO NANOMATERIALS: POTENTIAL EPIGENETIC MECHANISMS AND GENE EXPRESSION IN E. CRYPTICUS

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Abstract

Exposure to stressors can cause epigenetic changes, such changes are not detected when using the conventional testing methods for risk assessment (RA). In this study the epigenetic changes over multiple generations (MG), were studied. The standard test species Enchytraeus crypticus was exposed to soil spiked with copper oxide nanomaterials (CuONMs) and copper salt (CuCl2), 4 generations in spiked soil and 2 generations in clean soil (F1 to F7 generations in total). The exposure concentrations were population relevant (reproduction) test-effect concentrations, i.e. EC10, EC50. The global methylation and specific gene expression profiles were evaluated along generations, targeting genes involved in DNA methylation mechanisms, histone modifications and non-coding RNA. Results showed significant changes in gene expression, depending on both generation and Cu form. This is part of a collection of evidences that Cu may affect organisms via epigenetic mechanisms, and that there are nanoparticulate specific effects. Results highlight the importance of including multigenerational effects in the regulatory RA framework.

Keywords - Generational, Transgenerational, Gene regulation, Oligochaete

P29

METABOLOMIC PROFILING AND ENZIMATIC ACTIVITIES CHARACTERIZATION IN MEDITERRANEAN MUSSELS AFTER SULFAMETHOXAZOLE EXPOSURE

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Abstract

Sulfamethoxazole antibiotic is extensively used in human and veterinary medicine (including aquaculture) due to its bactericidal broad spectrum and low cost. The presence of this compound in the aquatic environment may result in a chronic exposure of marine organisms, especially those living in coastal areas like mussels. However, sub-lethal effects of these contaminants in marine organisms have been scarcely investigated. In this context, the analysis of target enzymatic activities and alterations in endogenous metabolites of mussels may provide further understanding of organism's response to this type of contamination.

In this work, a mesocosm experiment was undertaken where mussels (Mytilus galloprovincialis) were exposed to sulfamethoxazole (10 µg/L) during 96 hours. After the exposure period, mussels went through a depuration phase of 24 h under real commercial conditions. The experiment was performed during two seasons: summer (water temperature 16ºC) and winter (water temperature 12ºC). Enzymatic activities were measured in mussel's haemolymph, digestive gland and gills, besides, haemolymph was further analysed with HPLC-HRMS (LC-LTQ-Orbitrap) for metabolomics profiling. In addition, sulfamethoxazole concentration in mussels was also monitored using UPLC-MS/MS (UPLC-QqLIT) in order to relate real concentration in organism with effects.

Sulfamethoxazole accumulated in biota up to 12 ng/g dry weight after 96h of exposure. During the depuration period a 41% of clearance in the whole body was achieved after 24h. No significant differences were observed between the two studied seasons. A slight increase in glutathione reductase activity and glutathione S-transferase were detected in digestive gland after sulfamethoxazole exposure; whereas no changes were detected in carboxylesterase and catalase activities.

A metabolomics study was performed based on the non-target analysis of endogenous metabolites in haemolymph samples and the results were evaluated jointly with the bioaccumulation values and the other ecotoxycological parameters. This comprehensive and multi-disciplinary approach provided further understanding in sulfamethoxazole toxicology to marine mussels.

Keywords - Metabolomics, Antibiotic pollution, Marine mussels

P30

MODULATION OF LIPID METABOLISM IN THE DIGESTIVE GLAND OF MUSSELS BY ORGANOTIN AND PERFLUORINATED COMPOUNDS

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Abstract

The endoplasmic reticulum is involved in the metabolism of lipids and lipid-soluble compounds in eukaryotic cells, and it is the site of synthesis of several lipids, including phospholipids, di- and tri-acylglycerols (DGs, TGs), and cholesterol. Some pollutants, by interfering with lipid metabolism and inducing the intracellular accumulation of lipids, have been classified as obesogens in vertebrate models. Nonetheless, little is known on the interaction of pollutants with lipid dynamics in molluscs. Thus, this work aims at (a) characterizing the lipidome of digestive gland microsomal fractions of mussels using flow injection analysis coupled to high resolution mass spectrometry (FIA-HRMS); and (b) assessing the synthesis of lipids in digestive gland microsomal fractions incubated with ATP & CoA. FIA-HRMS analysis allowed the detection of more than 180 lipids in digestive gland microsomal fractions, including various types of phospholipids (phosphatidylcholines (PC), phosphatidylethanolamines (PE), PC- and PE-plasmalogens, phosphatidylserines (PS) and phosphatidylinositol (PI)), TGs and DGs. Incubation of microsomal fractions with ATP and CoA allowed the activation of endogenous fatty acids, and the synthesis of DGs (i.e. 36:6, 38:5), TGs (i.e. 52:6, 54:6, 56:6) and membrane lipids (PCs, PE, PI, PS) was evidenced and analyzed by partial least-square discriminant analysis. The addition of model chemicals to the incubation mixture led to significant changes in lipid synthesis. Thus, 10- 100 µM tributyltin (TBT) induced an accumulation of DGs (36:6, 38:6, 38:7, 40:6) and a concomitant decrease of TGs, together with a decreased of highly unsaturated PCs, while the presence of 100 µM perfluorooctanoic acid (PFOA) in the incubation mixture mainly decreased the synthesis of PCs, PS and PIs. Our findings suggest the usefulness of microsomal fractions isolated from key organs to be used as in-vitro models for the screening of environmental contaminants suspected to disrupt lipid metabolism.

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MAKING SENSE OF METABOLOMIC DATA: COMPREHENSIVE ANALYSIS OF ALTERED METABOLIC PATHWAYS IN DIABETES AND OBESITY

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Abstract

Bioinformatic analysis and visualization techniques for 'omics' data are key tools for understanding complex biological systems. They reduce the complexity of data and allow generating hypotheses and searching for disease biomarkers. The aim of this work is to analyse the suitability of bioinformatic tools to interpret metabolomics datasets of a range of diseases including type 1 and 2 diabetes and obesity.
We examined several disease datasets from metabolomics studies through different bioinformatic approaches: metabolic pathways, networks and disease-/functional-based analyses. Then we have analysed the accuracy of these tools to identify several traits of the datasets and their suitability to perform enrichment analyses.
The analysis of metabolic pathways, small-scale systems of biochemical reactions and events of regulation and signalling, proved to be the most appropriate approach to analyse metabolomics datasets. Tools based on KEGG metabolic pathways were the most suitable ones as they allowed us examining metabolic alterations in type 1 and 2 diabetes and obesity and formulating hypotheses about the physiopathology of these diseases. For instance, alterations in the metabolism of amino acids, nitrogen, glutathione, sphingolipids and primary bile acids were revealed in diseased conditions.
The study of altered metabolic pathways allowed us interpreting data from metabolomics studies and extracting very valuable information from them that might help identifying disease biomarkers and possible metabolic alterations related to diseases. This information could be translated to the clinical practice to predict metabolic alterations before the onset of diseases.

Keywords - Metabolomics, Bioinformatic, Enrichment analyses, KEGG, Pathways, Omics, Biomarkers

P32

ASSESSMENT OF THE RECOVER THE ORIGINAL NETWORKS IN GAUSSIAN GRAPHICAL MODELS

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Abstract

Gaussian Graphical Models (GGM) has recently become a popular tool to study association networks. Application of GGMs to omics data is quite challenging, as the number of variables (p) is usually much larger than the number of samples (n), and classical GGM theory is not valid in a small sample setting.
Several algorithms have been developed to handle GGMs with small samples. These algorithms boil the problem down to finding suitable estimates for the covariance matrix and its inverse when n < p. In this work, we have verified through simulations the ability of the methods to recover the original structure of direct interactions.
We have used different algorithms implemented in the R package GGMselect and significance tests of the partial correlation coefficient in the case n> p, adjusting p-values for multiple comparisons. In practice we have generated graphs of order p, we have obtained random samples of size n from the generated structure and we have verified if the original state: connected or not connected, is recovered between each pair of variables.
Results show, on one hand, a dependency on the ratio n/p. The greater the ratio, the better it fits. On the other hand, results depend only very slightly on the original network structure. Similar results have been obtained with highly structured networks or when the original structure is random.

Keywords - Keywords - Gaussian Graphical Models, Association Networks, Direct Association.

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