Session 3: (Eco)toxicological omics

14 | Network analysis predicts genes associated with silver toxicity in Chlamydomonas reinhardtii

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Although the toxicological processes induced by some chemical stressors have been characterized, toxicity pathways and mechanisms of action at the cellular level are still largely unknown. Integrative bioinformatics and statistical analyses of large and diverse collections of omics datasets can reveal the mechanisms of cellular response of aquatic organisms to chemical stressors. Specifically, gene co-expression network analyses, which are based on the guilt-by-association principle, can identify hubs and modules of genes with similar expression patterns, indicative for structured, modular toxicity response characteristic to certain chemical stressors. We developed netoxi – an integrative systems biology workflow, which infers mutual information co-expression networks from large collections of transcriptome data. netoxi uses both in-house generated omics datasets and publicly available data, and an entropy based mutual information metric is used to infer co-expression relationships between gene products. The resulting network is visualized as a graph. We built a co-expression network of Clamydomonas reinhardtii, based on 650 transcriptome samples in taken from public databases, including an in house transcriptome study of silver toxicity. While the mechanisms of silver uptake/transport/export are mostly unknown, they have been linked to copper homeostasis. The exact mode of action of silver is also unknown, but silver is known to replace copper in metalloproteins, which decreases their function, and is preferentially bound by metallothionein, which increases the intracellular availability of essential metals, such as copper. We searched the co-expression network around known copper transporters for genes that differentially expressed under silver exposure, or genes that connect copper transporters to copper responsive transcription factors, and came up with 17 genes with unknown functions, potentially involved in silver toxicity. We then measured the sensitivity of the respective 17 Chlamydomonas mutants to silver and discovered several had a significantly different EC50 to the controls. The elucidation of the molecular function of these is currently in progress.

15 | Ionising radiation induced stress responses in the macrophyte Lemna minor: comparison at different levels of biological complexity, from growth to gene expression.

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Nuclear energy production encompasses a variety of industrial activities, from mining and milling of fuel through to power generation and waste management and potentially release low amounts of radioactive substances to the environment many of which are long lasting gamma or beta emitters. In this study the floating plant Lemna minor was used as model system. Lemna plants were exposed for 7 days to either gamma radiation (delivered by an external Cs-137 source) or beta radiation (by addition of Sr-90 to the nutrient medium). In addition to growth (fresh weight, dry weight frond area and frond number), endpoints at different levels of biological complexity were studied such as photosynthesis, ploidy levels (via flow cytometry), antioxidative enzyme activity and gene expression were compared. Gene expression analysis was accomplished through an RNAseq assay and by assembling a draft genome of Lemna minor that could be used as a reference genome. It was found that both γ- and β-radiation significantly affected L. minor growth and development by triggering metabolic, physiological, and genetic reprogramming. After exposure to different IR dose rates two

distinct dose rate dependent phases in the response could be observed for which evidence was found at different levels of biological complexity. Dose rates levels from 0.09 mGy h-1 up to 232 mGy h-1 led to an adaption response towards IR including a reduction of the root length coupled with an activation of biosynthesis pathways of flavonoids. Dose rate levels of 423 mGy h-1 or higher triggered antioxidative systems and induced a growth stop with an increase in endoreduplication at 1500 mGy h-1. This means that from a certain dose rate, the L. minor plants induced a metabolic switch to cope with toxic levels of IR. This switch value lies between 233 mGy h-1 and 423 mGy h-1 for L. minor plants in the current set up. The findings in this study allow for a comparison of the sensitivty of L. minor towards gamma or beta radiation at different levels of biological complexity. The implications of these findings in terms of the potential mechanisms and predictive risk assessment will be discussed in this presentation. Acknowledgement - The authors thank the Research foundation-Flanders (FWO) (G.A040.11N) and the European Commission (Contract Fission-2010-3.5.1-269672 to STAR (www.star-radioecology.org)) for financial support.

16 | Using transcriptomics to understand crustacean intersexuality: untangling feminisation, demasculinisation, parasitic and environmental influences.

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The reproductive dysfunction associated with intersexuality in molluscs and vertebrates and is often a serious threat to ecosystems. However, intersexuality has also been found in many crustacean species and evidence indicates crustacean reproduction may also be vulnerable to anthropogenic pollutants, environmental change and parasitic manipulation. Detailing intersexuality-associated gene expression patterns has revealed the molecular changes that underlie vertebrate intersexuality and has given scientists and regulators powerful molecular markers for detecting evidence of feminisation and demasculinisation in vertebrates. To help environmental toxicologists monitor crustacean populations, we have produced a transcriptomic wide understanding of crustacean intersexuality by applying next generation sequencing technology to amphipods presenting various intersex phenotypes. We have revealed new links between intersexuality and both contamination and parasitism, and also revealed insights into the reproductive and immune responses of crustaceans infected with feminising parasites. Furthermore, we find that gene expression biomarkers of vertebrate feminisation are not suitable for monitoring crustaceans, as the orthologous genes are not induced in feminised amphipods. Critically, the broad-scale comparison of all reproductively-linked genes reveals that males retain the normal expression of male reproductive genes even when clearly feminised. This suggests extensive regulatory separation of the genes networks responsible for male and female characteristics and demonstrates that, unlike vertebrates, feminisation is not a proxy for demasculinisation in crustaceans. We suggest that widespread forms of crustacean intersexuality do not necessarily indicate serious sexual dysfunction and reveal a suite of tailored biomarkers to facilitate monitoring of crustacean reproductive health.

17 | Global transcriptional responses to repeated copper exposures in the three-spined stickleback (Gasterosteus aculeatus)

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Wildlife populations are affected by chemical pollution in their habitats throughout life. Exposures can occur continuously over long periods of time, but more frequently they occur at relatively high concentrations for short durations (e.g. during intense rainfall or as a result of chemical spills) followed by periods of relatively low exposure. The ability of fish to respond to chemical exposures may, therefore, be determined by their exposure history, and previous exposures to a stressor may influence the ability of a population to respond to subsequent exposures. Despite this, little is known about the impacts of historical exposures on an individual's susceptibility to exposures in the future. We aimed to address this question using the stickleback as a model fish species due to its amenability to toxicological studies, its widespread range in temperate aquatic systems and the availability of genome sequence information, facilitating transcriptomics and epigenomic studies. Copper was chosen as a model chemical because it is widespread in aquatic environments and its toxicological effects in fish, including at the transcriptomic and physiological level, are well described. We exposed adult males to 0 or 0.02 mg Cu/L for 4 days, followed by a period of depuration for 4 weeks. After this period, fish were re-exposed to either 0 or 0.02mg Cu/L for a further 4 days and tissues were collected for analysis of copper content and global transcriptomic analysis using RNA-Seq (on an Illumina Hi-Seq 2500 platform). Following copper exposure, we observed an increase in copper uptake in fish pre-exposed to copper compared to naïve fish. In addition, we found significant alterations in the transcriptomic responses to copper in pre-exposed fish compared to naïve fish, suggesting that previous exposure modulates the response to copper upon subsequent exposures in the stickleback. We are now investigating the mechanistic bases of these differences. Together, our data suggest that management of fish populations in polluted environments should consider the influence of exposure history on the susceptibility of fish to future exposures in their environment.

18 | The Value of (Eco)Toxicogenomics Realized: From Zero to Validated Adverse Outcome Pathway (AOP) with Genomics Guiding the Way

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Genomics investigations served as the foundation for discovery of a critical adverse outcome pathway (AOP) with relevance to both ecological and human health. The work represents a suite of studies that established the molecular, mechanistic and systems-level impacts of the most abundantly distributed class of munitions contaminants, the nitrotoluenes. Historical toxicological investigations of nitrotoluenes identified lethargy, weight loss and muscle wasting as general adverse outcomes (AOs) of exposure. In order to identify the source(s) of these AOs, global-transcriptomics expression investigations of nitrotouene exposures were conducted to identify the genes and pathways affected. A recurring theme of inhibited expression for transcripts involved in lipid and systemic cellular-energy metabolism pathways were observed in multiple species including rats, mice, birds, and fish in response to various nitrotoluenes. Effects on these pathways were confirmed in proteomics and lipidomics investigations where lipid catabolism was impaired leading to fatty acid accumulation in the liver in conjunction with muscle wasting and overall weight loss. An additional recurring omics response to nitrotoluenes was significant enrichment of peroxisome proliferator-activated receptor (PPAR) signaling pathways where expression of component genes were strongly inhibited. PPARs represent key transcriptional regulators of pathways controlling systemic energy metabolism, where the PPARα isoform controls various facets of lipid transport and metabolism. We then tested the effects of nitrotoluenes on PPARα signaling in human in vitro nuclear receptor

inhibition assays confirming that various nitrotoluene structures caused significant signaling inhibition. Given the knowledge above, we developed a hypothetical AOP establishing PPARα binding / inhibition as the molecular initiating event (MIE) for a series of key events leading the adverse outcome (AO) of overall starvation-like weight loss. The connection between impaired PPARα signaling and the AO was validated in PPARα knockout (K/O) mice where the PPARα K/O eliminated the AO resulting from nitrotoluene exposures. Computational docking assays support the putative MIE indicating probable binding of nitrotoluenes to the PPARα active site, a hypothesis presently being tested in PPARα competitive-binding assays. Overall, this work demonstrates the power of omics techniques for leading systems-level development of AOPs.

19 | Towards developing a Framework for transcriptomics and other Big Data analysis for regulatory application

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Molecular events associated with the effects of chemical, biological and physical agents in biological systems can be globally determined using ‘omic high throughput technologies. These technologies are playing a major role in the generation of new knowledge and understanding in mechanisms of toxicity. The use and application of these technologies has however been slower in hazard identification and regulatory risk assessment. This is partly because from the first generation of these data sets it was apparent that, even before considering interpretation, large data sets pose challenges. Some of these challenges have been quality control of data generation, normalization, recognition of outliers and univariate statistical analysis. Additionally there are challenges with the associated experimental meta data and last but not least data interpretation. There are biological and experimental variables revealed by these large data set that may not be seen, or be of consequence, when fewer measurements are taken. This presentation will discuss these challenges (data analysis standard, meta data, quality control, etc.) and their implication in regulatory application. For example, why the adoption of standards for the univariate data analysis has been slower than the adoption of the standards for meta data collection and standardisation of methods for the generation of data. The causes for this are not clear but one possible reason is that there are many different ways of processing the data. Everyone has their favourite and can divide the data in the way that suits their experiment or hypothesis for example by changing the statistical parameters. While this can be acceptable for research where justification for the method used will be subject to peer review and likely replication, it is not acceptable for regulatory use where consistency is paramount. While certain mathematical and statistical methods for the univariate have achieved a level of greater acceptability, a framework of best practice has not been developed that can be routinely applied to the primary analysis of data to the point of the generation of a gene list for subsequent interpretation. This presentation will outline this issue and present the initial thoughts from a group of experts convened to examine the issues under the auspices of ECETOC.

20 | SETAC Global Advisory Group OMICS: Cutting-edge science to solve real world problems

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This SETAC Global Advisory Group (AG) was established in 2015 and aims to coordinate scientific efforts by building bridges between different stakeholders. The group explores OMICs technologies as an important feature in weight-of-evidence-based risk assessment and for environmental monitoring. The goal is to improve decision making in hazard and risk assessment by means of providing additional lines of evidence to conventional and novel methods, and to facilitate the innovation of ethical and cost-effective testing procedures. This AG is a key player in a global dialog, cementing a leading role for SETAC in fostering ongoing discussions about how to proceed on a scientific level to maximize gains for industry and policy makers around the world. The immediate objectives of this AG include, but are not limited to (i) explicitly defining indispensable tools for predictive toxicology and effective risk management (ii) working towards validation, standardisation and guidelines proposal on the use of these techniques (iii) linking molecular events to organismal and ecological adverse outcomes (iv) planning a workshop for developing coordinated case studies that provide proof-of-concepts for clear areas of application.

21 | Delayed impacts of developmental exposure to 17-α-ethinylestradiol on the phenotype and the proteome of the self-fertilizing fish Kryptolebias marmoratus

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17-α-ethinylestradiol (EE2) is one of the most potent endocrine disrupting compounds found in the aquatic environment, and is known to strongly alter fish reproduction and fitness. While the effects of EE2 are well studied in adults, impacts of developmental exposure remain largely uncharacterized. Our study investigated the adult's cellular (proteome) and organismal phenotypic outcomes of early-life exposure to EE2 in the mangrove rivulus, Kryptolebias marmoratus. Being one of the only two self-fertilizing hermaphroditic vertebrates, rivulus makes it possible to work with genetically identical individuals and exclude the effect of genetic variance on the phenotype. We exposed hatchlings during 28 days to 0, 4 or 120 ng/L of EE2. They were then reared in uncontaminated water until 168 dph (days post hatching). Growth, egg laying and steroid hormone levels (estradiol, cortisol, 11-ketotestosterone, testosterone) were measured throughout development. Then, the proteome was analyzed in gonads, brain and liver of 168 dph adults. Exposed fish showed a reduction in standard length directly after exposure (28 dph), which was more pronounced in the 120 ng/L group. This was followed by compensatory growth when reared in clean water. At 4 ng/L, fish layed significantly fewer eggs than controls, while, surprisingly, reproduction was not affected at 120 ng/L. There were no effect of 4 ng/L on steroid hormones, but 120 ng/L treated fish exhibited significantly higher levels of testosterone at 91 and 168 dph and 11-ketotestosterone at 168 dph, or about 140 days after the exposure ceased. This suggests that at 120 ng/L, compensatory mechanisms could allow to maintain reproduction. To gain mechanistic insight into the effects of EE2, shotgun proteomics was performed on gonads, brain and liver of 168 dph adults. Of all 3 organs, the liver showed the most interesting protein profiles. Differencially expressed proteins in the liver were confirmed by targeted proteomics and are inlvolved in the regulation of histones, protein translation and folding, fatty acid metabolism and energy metabolism. The majority of these proteins were differencially regulated in the 4, but not in the 120 ng/L treatment compared to control. Our study demonstrates that developmental EE2 exposure can impact both the cellular and organismal phenotypes of adults. Whether these effects

have the potential to impact the fitness of an individual's lifetime, or future generations remains to be elucidated.

22 | Shotgun proteogenomics & multiplexed targeted proteomics define robust, specific protein biomarkers from non-model species for environmental monitoring

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Biomarkers related to exposure to chemical compounds can be monitored to predict possible hazard on health of sentinel organisms. The routine use of these tools in biomonitoring faces several drawbacks, especially in invertebrates: the lack of robust species-specific quantification methods and the use of numerous biomarker-specific protocols that lead to very expensive biomonitoring strategies in time, cost and biological samples. Recently, "proteogenomics" emerged as a relevant strategy for the discovery of proteins in non-model organisms. With the combination of high throughput genomic and proteomic methodologies, Trapp et al. built a protein sequence database consisting of 1873 specific proteins of the amphipod crustacean Gammarus fossarum, a species of interest for freshwater biomonitoring. Based on this protein database, the objective of the present study was to develop an approach that allowed a fast, specific and simultaneous quantification of multiple proteins of interest in this species. Following recent methodologies for the development of biomarkers in human disease diagnosis, we implemented a quantitative multiplexed targeted proteomics assay (using Selected Reaction Monitoring mass spectrometry) to study 55 protein biomarker candidates. Identification of specific proteotypic peptides and assessment of their interest as biomarkers in G. fossarum were achieved by following their quantitative changes through male and female reproductive cycles and after exposure to stresses, such as food privation and exposure to contamination through laboratory and in situ (caged organisms) assays. The levels of 21 biomarkers of interest (sex-specific proteins and/or with key physiological functions) were simultaneously monitored in several biological samples during their physiological processes. Their sensitivity to toxic contamination was demonstrated both in laboratory and during field monitoring. For example, the laboratory contamination with environmental concentrations of cadmium modulated the expression levels of some biomarkers annotated as the Na+K+ATPase (cellular pump), catalase (oxidative defense) and GST (detoxification) proteins. This breakthrough methodology in ecotoxicology offers a valid alternative to the currently used protocols, paving the way for future practical applications of protein biomarkers in chemical risk assessment and environmental monitoring.

23 | Is protein carbonylation a sensitive effect marker of gamma irradiation damage? Comparison between acute and chronic irradiation

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Non-human species are subjected to ionizing radiation chronic exposure, but involved toxicity mechanisms remain unclear. Indeed, for a same cumulated-dose, the observed effects are different after acute and chronic-radiation, meaning differential underlying mechanisms. After a huge acute dose of gamma rays (>200Gy), it has been demonstrated that differential radiosensitivity between species could be explained by a differential proteome protection particularly against oxidation, i.e. carbonylation (Krisko and Radman 2010). The aim of this work is to better understand the role of carbonylated protein (CP) induction and degradation (by cellular defense such as proteasome) in the acute vs chronic effects induced by gamma irradiation. C.elegans were exposed to 3 cumulated doses (3 – 6 – 12 Gy) obtained either by chronic or acute exposure to 6 different dose rates. Reproduction was then monitored by measuring the cumulated number of larvae. Rate of CP have been investigated after direct hydrazide coupled fluorophore derivatization of carbonylated proteins and signal analysis. Nature of carbonylated protein has been investigated after derivatization and a modified proteomic approach by two dimensional electrophoresis followed by signal analysis. Spots of interest were analyzed by mass spectrometry. After chronic irradiation, significant decrease of reproduction is observed from 3Gy; on the contrary, no effect is observed after acute exposure. The study of CP rate after acute irradiation at 6 and 12Gy showed respectively 1.2 and 2 fold induction compared to controls, but not after chronic irradiation. These results were confirmed by the study of carbonylomes by 2D analysis. After chronic irradiation at 3Gy, 29 different carbonylated protein spots between controls and exposed were found, whereas after acute irradiation, 113 different carbonylated protein spots differ and 15 have been identified by mass spectrometry. These results are particularly interesting as the results are different between acute and chronic, meaning that CP could be an early marker of acute irradiation effect. To check this hypothesis and to know if CP is a sensitive marker, comparison of equivalent doses inducing damages will be studied. In addition, as protein is the functional unit of cells (DNA repair enzymes, proteasome sub-units), CP nature study as well as proteolytic activity of cells will help to improve the knowledge on radiation-induced toxicity molecular mechanisms. Acknowledgements: Dag Brede NMBU Oslo-Norway.

24 | Using an integrative OMICs approach to unravel Glyphosate mechanisms of toxicity in Folsomia candida

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The application of molecular tools to evaluate environmental health status is becoming increasingly important in soil management and risk assessment. Collembolans have been traditionally used as soil health indicators with Folsomia candida representing one of the most commonly used “standard” test organisms for estimating the effects of pesticides and other environmental pollutants in soil organisms. To further understand the mechanisms of toxicity behind pesticide contamination it is important to complement such information with responses at lower levels of biological organization. The “Omics” approach provides adequate and pillar techniques for such holistic understanding of the interaction stressors vs organisms. The present work aims to address the toxicity mechanisms of glyphosate, still one of the most widely used herbicides in agriculture worldwide, on F. candida by establishing the link between the effects on reproduction with gene expression and protein levels, simultaneously. For survival and reproduction tests, the organisms were exposed to a commercial formulation of glyphosate (Montana®, 30.8% active ingredient) in a natural agricultural soil, following

the ISO 11267:1999 guideline. Organisms were then exposed for 10 days to the reproduction EC50 (4.95 mg a.i./kg) of the pesticide, with sample collection at days 4, 7 and 10. Protein and RNA were extracted from the same organisms using TRIZOL® reagent. RNA sequencing was applied to assess differential gene expression between exposed and control organisms, at the several time points. Shotgun proteomics based on liquid chromatography and tandem mass spectrometry (LC–MS/MS), combined with iTRAQ labelling were used to identify differential protein levels between exposed and control organisms. Different gene sets and gene expression patterns were observed a long time, with a higher number of genes and biological processes being affected at the longer exposure period. Gene Ontology enrichment analysis revealed that Glyphosate affected fatty acid metabolism, chitin metabolism, vitelline membrane formation and proteolysis. Interestingly, some of the responses observed at the gene level could also be confirmed at the protein level. For example, vitellogenin proteins and fatty-acid binding proteins were found to be significantly affected. This work constitutes the first attempt to understand the toxicity mechanisms underlying the effects of glyphosate in soil invertebrates and highlights the usefulness and importance of an integrative OMICs approach to obtain more causal information on adverse effects caused by herbicides in non-target organisms.

25 | Multi-species metabolomics to enable the design of cost-effective biomonitoring programs for determining the impacts of pollution in estuarine environments.

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Measuring biological responses in resident biota is a commonly used approach to monitoring polluted habitats. The challenge however, is to choose taxa that reflect the sensitivity of the ecosystem, whilst avoiding the most sensitive or tolerant organisms which could lead to an under- or over-estimation of the level of risk to the whole ecosystem. Collection and identification of specific species requires taxonomic expertise and the time and resources required to complete this is impractical for large biomonitoring programs. Therefore, alternative approaches are needed, ideally ones where the biological response to be measured is both sensitive and stressor-specific. Metabolomics is a potentially useful approach for identifying sensitive and consistent biomarkers in organisms following exposure to chemicals, and due to the holistic nature of this technique, it is also valuable in identifying metabolic pathways that are affected by toxicants. Here we use metabolomics to characterise the responses of multiple species to a suite of chemical stressors commonly detected in estuarine environments, and determine whether responses of individual species reflect responses of a range of taxa. Macroinvertebrate taxa, including polychaetes and gastropods, were collected from the intertidal zone of an estuary in Victoria, Australia. Individuals were exposed to two concentrations of stressors pertinent to estuarine environments, including metals, fungicides, nutrients and salinity. Concentrations were based on individual species' responses to each chemical (i.e. LC10 values) and also to Australian water quality guideline (trigger) values. If no guideline values were available, species were exposed concentrations that have been routinely detected in Victorian estuaries. Therefore all individuals were exposed to a sublethal concentration as well as the same, environmentally relevant test concentration, so that differences in sensitivity between taxa could be determined. Whole body homogenates of each species were extracted, then a multi-platform metabolomics approach (untargeted GC/MS for global changes in polar metabolites and targeted LC/MS to measure changes in amine-containing metabolites) was used to determine effects on a wide range of biochemical pathways. Suitable tissue preparation methods were established and key metabolites were identified, which will be discussed in the context of suitability of incorporation into future biomonitoring programs.

26 | Peptidomics of the zebrafish: discovering neuropeptides by LC-MS

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(Neuro)peptides are small messenger molecules that are derived from larger, inactive precursor proteins by the highly controlled action of processing enzymes. These biologically active peptides can be found in all metazoan species where they orchestrate a wide variety of physiological processes. Obviously, detailed knowledge on the actual peptide sequences, including the potential existence of truncated versions, is of high importance when studying their function. A peptidomics approach therefore aims to identify and characterize the endogenously present peptide complement of a defined tissue or organism using liquid chromatography and mass spectrometry. While the zebrafish Danio rerio is considered as an important aquatic model in ecotoxicology or rather as general vertebrate model in a medical context, very little is known about their peptidergic signaling cascades. We therefore set out to biochemically characterize endogenously present (neuro)peptides from the zebrafish brain. Our peptidomics setup yielded > 50 different neuropeptides in addition to various truncated versions. This archive of identified peptides is likely to aid future research in (neuro)endocrinology in this important model organism. As the endogenous peptide content of a cell, tissue or organism is spatially and temporally dynamic, we aim investigate which signaling cascades are affected upon exposure to environmental stressors by adopting a differential peptidomics approach or by using molecular imaging techniques such as MALDI imaging in the furture.

27 | An integrated 'omics approach to elucidate low-dose effects of xenobiotics in zebrafish larvae (Danio rerio)

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Morphology-based toxicity assays such as the OCED Zebrafish Embryo Toxicity Assay TG236) allow correlation of chemical exposure to adverse phenotypes. However, in order to accommodate large scale screening, and to expedite environmental monitoring efforts, a faster, reliable, and more sensitive method is desirable. Integration of multi-omic response measurements to low-dose xenobiotic exposures provides unprecedented potential in enhancing prediction of toxicological outcomes. In the current study, we assessed the toxicological relevance through alterations of the metabolome and parallel transcriptomic responses in zebrafish larvae (ZF; Danio rerio) acutely exposed to environmental concentrations of acetaminophen (APAP), diphenhydramine (DH), carbamazepine (CBZ), and fluoxetine (FLX); common pharmaceuticals with known modes-of-actions (MOAs). Perturbations in the qPCR-based transcriptome and targeted metabolome were measured following exposure of ZF larvae during the 96-120h post-fertilization period. Transcriptomic and metabolomic changes were evaluated and integrated to identify molecular interactions and biological relevance of the responses. Results indicated chemical and dose-specific changes suggesting differences in the time scale of transcript turnover and metabolite production. Increased metabolomic response relative to transcript changes in FLX-treated animals suggests a stronger post-

translational effect of the treatment. In contrast, the transcriptome showed higher sensitivity to perturbation in DH-exposed animals. Integration of 'omic responses using multivariate approaches provided additional insights not obtained by independent 'omic analyses and demonstrated the most distinct overall response profiles were induced by the low dose groups in all 4 pharmaceuticals. Changes in transcript abundance corroborated predictions from metabolomic enrichment analyses and aligned with known MOAs of the xenobiotics. For example, the predicted disruption of leukotriene metabolism and prostaglandin formation from arachidonates in DH-exposed animals was supported by changes in Ω6 fatty acid concentrations and il6 and PPARaa transcript abundances. As demonstrated, multi-omic integration coupled with a sensitive toxicological model, such as ZF larvae, can facilitate a systems biology approach in identifying and characterizing the toxicological relevance of acute low-dose exposures.

28 | Addressing a key challenge in non-targeted environmental metabolomics: identifying the parts list through Deep Metabolome Annotation

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Non-targeted metabolomics is now widely applied across fields from medicine and toxicology to ecology and agriculture, with the goals to discover and help to characterise the molecular mechanisms associated with biological processes. The field of environmental metabolomics is uniquely challenging due to the vast metabolic diversity of the organisms being studied – from plants and animals to microbes – and due to the chemical complexity of the natural environment (the exposome). My research team has developed and applied non-targeted metabolomics approaches, including both NMR spectroscopy and mass spectrometry, for the past fifteen years. During this time the field has evolved relatively rapidly, yet many challenges remain, not least our ability to identify the large numbers of metabolites detected in metabolomics studies. In this presentation I will highlight some of the unique challenges of environmental metabolomics and then describe some examples of the approaches used to identify endogenous metabolites (and exogenous pollutants). None of these methods, however, have transformed our ability to identify the metabolome. This more-than-a-decade long challenge has motivated my team to embark on a new endeavour, termed Deep Metabolome Annotation, in an attempt to characterise the metabolic biochemistry of organisms (beginning with *Daphnia magna*, the water flea) much more comprehensively than has ever been attempted previously. Such deep annotation is currently limited in its application to a small number of species because of the time and resources needed. Therefore I will conclude my talk by making the case that the time is now right to focus on the deep annotation of metabolites in a select few model organisms.

29 | Epigenetic basis of honeybee development, behavior and health

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Honeybees are essential pollinators and possess a unique biology that facilitates the study of phenotypic plasticity and complex behaviors. While toxins and parasites threaten this important pollinator, new knowledge of the honeybee genome allows us to study the impact of environmental factors on the honeybee at a molecular level. This environmental impact may be mediated by epigenetic mechanisms, which have recently been shown to integrate hive cues to direct development in larvae and task switching in adults. Epigenetic mechanisms allow for flexible and temporal control over gene expression through the placement of chemical tags at the level of DNA itself. These epigenetic tags assist in caste development and the task switching in the hive by integrating nutritional and social cues of the hive to refine the phenotype of individual bees in the

hive. Here we explore the role of epigenetics in the development and organization of the hive, and relate this to the possible effects of environmental factors threating this species. Special attention will be given to performing genomics, epigenomics, and bioinformatics work in an emerging organism.