8th International Plant Chemical Biology Conference
Focus on Physical Chemistry

Syngenta Jealott's Hill International Research Station
Berkshire, UK
Thursday 13th April, 2019

AGRI-net

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8th International Plant Chemical Biology Conference
Focus on Physical Chemistry

Syngenta Jealott's Hill International Research Station, Berkshire, UK, RG42 6EX

Thursday 13th June 2019

10:00 Registration, poster viewing plus tea & coffee

10:25 Opening Remarks: Andrew Crossthwaite - Syngenta

Session I – Chair: Andrew Crossthwaite - Syngenta

10:30 Maddalena Bronzato - Syngenta
Measuring the interplay between uptake and loss processes of xenobiotics

11:10 Oscar Ces - Imperial College London
Novel Biomembrane Technologies for the Agrisciences

11:50 Douglas Kell - University of Liverpool
No transporters means no transport – and assessment of the 'real' (natural) substrates of xenobiotic transporters.

12:30 Lunch, networking & poster session

Session II – Chair: Chris Baker - Syngenta

13:50 John Misselbrook - Agform Limited
Nanotechnology in Agrochemicals – Challenges and Opportunities

14.10 Rob Law - Imperial College London
Some Investigations into Model Soil Systems

14.50 Syma Khalid - University of Southampton
Focusing the Computational Microscope on the Cell Envelopes of Gram-negative Bacteria

15.20 Closing remarks: Rudiger Woscholski – Imperial College London

15:30 Conference closes

Organising committee:
Christopher M. Baker - Head of Physical Chemistry, Syngenta
Andrew Crossthwaite – Head of Insecticide Bioscience, Syngenta
Rudiger Woscholski - Co-director of Agri-net, Imperial College London
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ORAL ABSTRACTS

Session I

Maddalena Bronzato - Syngenta
Measuring the interplay between uptake and loss processes of xenobiotics
We have developed an assay that utilises a leaf substrate alongside a glass substrate. Processes such as volatility and photostability are important for understanding biological efficacy, environmental fate and registrability. Established methods which evaluate volatilisation and photodegradation on glass slides undeniably provide invaluable information, but the use of impermeable substrates such as glass inevitably prevents an assessment of their critical interplay with the biokinetics of foliar uptake.
From a biological efficacy standpoint, volatile movement can be considered a useful redistribution mechanism and vapour activity has been observed for fungicides (particularly mildewicides), aphicides and also pre-emergence herbicides. Photodegradation can lead to a loss of activity and persistence of effect but can be mitigated by rapid and extensive uptake. To understand the balance between abiotic loss processes and biological surface interactions we have developed an assay that utilises a leaf substrate alongside a glass substrate.

Oscar Ces - Imperial College London
Novel Biomembrane Technologies for the Agrisciences
This talk will outline novel biomembrane engineering strategies that are capable of fabricating vesicles, multisomes and artificial tissues on demand where parameters such as membrane asymmetry, membrane curvature, compartment connectivity and individual compartment contents can be controlled. These platforms are being exploited to generate (i) minimal biological models that are being used to study fundamental biological processes (ii) screening platforms for studying small-molecule membrane interactions (iii) artificial cells for smart delivery purposes and (iv) biological tissues. This will talk will give an overview of the fabrication technologies and associated downstream applications including those in the agri-sciences.

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Douglas Kell & Steve O'Hagan - University of Liverpool

No transporters means no transport – and assessment of the 'real' (natural) substrates of xenobiotic transporters.

Transporters have important roles in both cellular drug uptake [1] and biotechnology [2]. Despite what it says in textbooks, bilayer flux through intact cell membranes is negligible and hydrophobicity is often a poor guide to permeability. I'll give a couple of examples. Often their 'natural' substrates are unknown. We compare several molecular fingerprint encodings for marketed, small molecule drugs, and assess how their rank order varies with the fingerprint in terms of the Tanimoto similarity to the most similar endogenous human metabolite as taken from Recon2 [3]. For the great majority of drugs, the rank order varies very greatly depending on the encoding used, and also somewhat when the Tanimoto similarity (TS) is replaced by the Tversky similarity [4-8]. However, for a subset of such drugs, amounting to some 10% of the set and a Tanimoto similarity of ~0.8 or greater, the similarity coefficient is relatively robust to the encoding used. This leads to a metric that, while arbitrary, suggests that a Tanimoto similarity of 0.75-0.8 or greater genuinely does imply a considerable structural similarity of two molecules in the drug-endogenite space. Although comparatively few (<10-15% of) marketed drugs are, in this sense, robustly similar to an endogenite, there is often at least one encoding with which they are genuinely similar (e.g. TS > 0.75). This is referred to as the Take Your Pick Improved Cheminformatic Analytical Likeness or TYPICAL encoding, and on this basis some 66% of drugs are within a TS of 0.75 to an endogenite [9].

We next explicitly recognise that natural evolution will have selected for the ability to transport dietary substances, including plant, animal and microbial 'secondary' metabolites, that are of benefit to the host. These should also be explored in terms of their closeness to marketed drugs. We thus compared the TS of marketed drugs with the contents of various databases of natural products. When this is done, we find that some 80% of marketed drugs are within a TS of 0.7 to a natural product, even using just the MACCS encoding. For patterned and TYPICAL encodings, 80% and 98% of drugs are within a TS of 0.8 to (an endogenite or) an exogenous natural product. This implies strongly that it is these <u>exogenous</u> (dietary and medicinal) natural products that are more to be seen as the 'natural' substrates of drug transporters (as is recognised, for instance, for the solute carrier SLC22A4 and ergothioneine [10; 11]). This novel analysis [9] casts an entirely different light on the kinds of natural molecules that are to be seen as most like marketed drugs, and hence potential transporter substrates, and further suggests that a renewed exploitation of natural products space [12] as drug scaffolds (or indeed inhibitors) would be amply rewarded. The heterogeneous uptake is due to the enormous heterogeneity of transporter expression [13].

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[3] Thiele, I.,…Kell, D. B., Mendes, P. & Palsson, B. Ø. (2013). A community-driven global reconstruction of human metabolism. *Nat Biotechnol*. **31,** 419-425.

[4] O'Hagan, S., Swainston, N., Handl, J. & Kell, D. B. (2015). A 'rule of 0.5′ for the metabolite-likeness of approved pharmaceutical drugs. *Metabolomics* **11,** 323-339.

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[7] O'Hagan, S. & Kell, D. B. (2016). MetMaxStruct: a Tversky-similarity-based strategy for analysing the (sub)structural similarities of drugs and endogenous metabolites. *Front Pharmacol* **7,** 266.

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[12] O'Hagan, S. & Kell, D. B. (2018). Analysing and navigating natural products space for generating small, diverse, but representative chemical libraries. *Biotechnol J* **13,** 1700503.

[13] O'Hagan S, Wright Muelas M, Day PJ, Lundberg E, Kell DB: GeneGini: assessment via the Gini coefficient of reference ''housekeeping'' genes and diverse human transporter expression profiles Cell Syst 2018; 6:230-244.

Session II

John Misselbrook - Agform Limited
Nanotechnology in Agrochemicals – Challenges and Opportunities
The production and stabilisation of active ingredients in the sub-micron particle range has been a challenging field of research for a number of years. The recent commercialisation of agrochemical formulations containing stable particles in the nanoparticle range has demonstrated a number of advantages over conventional suspension concentrate formulations in respect to their efficacy and beneficial effects on crop yield. Furthermore, the technology has allowed the reduction in use rate of certain actives by up to 5 fold, with the attendant reduction in environmental impact of such actives. The technology is applicable to many compounds with aqueous solubilities of less than 100 ppm, although the benefits of the smaller particle size and resulting increase in the rate and degree of solubility does not always lead to improved biological properties.

Rob Law - Imperial College London
Some Investigations into Model Soil Systems
The role of carbon in soil is poorly understood, but is critical its long-term fertility and its ability to continue with this function. Here, we show the application of spectroscopy into the understanding carbon, in the form of lignin, of model soils. Model soils are based on a clay - silica -lignin and their mutual interactions are investigated by a variety of spectroscopy techniques but the main focus will be the utilisation of Magic Angle Spinning Nuclear Magnetic Resonance Spectroscopy (MAS NMR). These results are correlated with mechanical testing of soils.

Syma Khalid - University of Southampton
Focusing the Computational Microscope on the Cell Envelopes of Gram-negative Bacteria
A protective cell envelope surrounds Gram-negative bacteria such as *E. coli,* providing a selective gateway connecting the interior of the cell to the external environment. We employ molecular dynamics simulations, at multiple levels of resolution, to reveal the details of interactions between proteins, lipids, and sugars within the cell envelope to gain insights into how these molecules function individually, and also together as a whole. In the past, we have been able to use our models to study permeation of drug-like molecules across bacterial membranes (it is not as straightforward as simple phospholipid bilayers). We use data such X-ray, NMR and cryoEM structures from our structural biology colleagues, and combine them with molecular models that represent the *in vivo* environment of the bacterium to study their dynamics. More recently we have built an atomistic model of the *E. coli* cell wall, and have explored the interactions of inner and outer membrane proteins with the peptidoglycan that comprises the wall- we are now looking at how antibiotics diffuse through the cell wall and will soon be exploring how the cell wall responds to osmotic pressure. Our models can easily be extended to other species and we welcome the opportunities to work with experimental colleagues from a range of disciplines.
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POSTER ABSTRACTS

POSTER 1

Leah Taylor-Kearney - University of Oxford

A Study of Physiological and Molecular Responses to Hypoxia in Algae and Early Plants

All aerobic organisms require oxygen for survival. When oxygen is limited (hypoxia), a hypoxic response is required to reduce demand and/or improve supply. Plant hypoxia is typically due to reduced oxygen diffusion as a result of submergence or waterlogging, or as a result of barriers between internal organs (in seeds and gonads). While plants can survive temporary periods of hypoxia, prolonged exposure can result in plant damage or death. Due to a global concern over food security, plant hypoxic research thus far has focused on higher land plants, with little contributed to understanding marine or early land plants. The photosynthesis of algae and cyanobacteria is by far the dominant global process that replenishes atmospheric and oceanic oxygen, contributing 50 to 85 % of the oxygen in Earth's atmosphere. In light of the climate-change driven warming of our oceans, the decreased dissolution of oxygen with warming, and the reported expansion of oceanic oxygen minimums, the impact oceanic deoxygenation will have on algae, or vice versa, must be assessed. This study uses a multidisciplinary approach to understand the basal plant hypoxic response, employing recombinant protein studies, substrate identification and synthesis, novel culturing techniques and proteomics to elucidate the early plant hypoxic response and its evolutionary context.

POSTER 2

Chun Liu - Syngenta

Computer-based herbicide resistance modelling and sustainable weed management

The evolution of herbicide-resistant weeds has become an increasing challenge for world agriculture. For example, simplified farming systems based on glyphosate-tolerant crops and a single herbicide is not sufficient for protecting yields. More diversity in chemical and non-chemical control tactics is required for effective weed control. However, it is difficult to explore the multiple levels of complexity involved in these diversified programs using conventional experimental methods. Computer-based models are powerful tools for studying the dynamics of weed populations and evaluating the sustainability of weed control strategies. We have developed a range of herbicide resistance models of key weed species to investigate best management practices and promote sustainable use of herbicides. We will introduce the model design and an interactive user interface as a powerful educational tool. A few case studies will be presented to discuss the respective sustainability of stacked herbicide-tolerant traits vs. residual herbicides, the consequences of reduced application rates and the economic implications of resistance.

POSTER 3

Javier A Miret - University of Reading

Abscisic acid signalling manipulation suppresses senescence of a leafy vegetable stored at room temperature

Postharvest senescence limits the shelf life and nutritional value of vegetables. After harvest, controlled exposure to abiotic stresses and/or exogenous phytohormones can enhance commercial longevity, nutraceutical and organoleptic traits. Abscisic acid (ABA) contents rise with organ senescence progression, but its actual biological functions at the onset and through senescence still need to be clarified.

Postharvest senescence of green cabbage leaves (Brassica oleracea var. capitata) was characterized under cold (4 °C) and room temperature (25 °C) storage, Cytokinins decrease early under both conditions, while ABA only progressively increased at 25°C. ABA and a partial agonist of ABA (pyrabactin) increased cell integrity and altered 1-aminocyclopropane-1-carboxylic acid (ACC) and cytokinins contents together with the transcriptional regulation of ABA, cytokinin and ethylene metabolism and signalling. Senescence promoters were transcriptionally supressed; with a protection of chloroplast functionality and cell homeostasis; and suppression of defence responses (including glucosinolates and phenylpropanoids metabolism).

Thus, the early increasing of the concentration of ABA (or its partial agonist pyrabactin) can suppress senescence of stored leaves, change the transcriptional regulation of glucosinolates metabolism and down-regulate biotic stress defence mechanisms. These results suggest a potential for ABA signalling manipulation to improve the quality of leafy vegetables stored at ambient temperature.

POSTER 4

Nathan Hawkins - Anatune Ltd

SIFT-MS: The Gold Standard for Volatile Biomarker Verification and Validation?

Volatile organic (and inorganic) compounds are a universal in chemical communication within and between virtually all living species, are integral to carbon flux through the biosphere and a significant source of atmospheric pollution and greenhouse gas emissions. Since they are generally present at low, sub-ppm(v) concentrations in the atmosphere, their analysis is primarily done by GC-MS with preconcentration onto, for example, thermal desorption tubes. Whilst GC-MS is the gold-standard analytical platform for characterising complex mixtures (e.g., volatile biomarker discovery), getting reliable measurements is challenging:

- Analyte reactivity and stability – analysis as soon as possible after sampling
- Adsorptive losses of analyte in the analytical sample path
- Management of sample humidity (GC's are immiscible with water)

In this poster, we present a new technology, Selected-Ion-Flow-Mass-Spectrometry (SIFT-MS), for volatile biomarker validation and verification that features:

- Realtime analysis of volatile inorganic and organic species to single digit ppt(v)
- Speciation of complex mixtures, and structural isomers, without chromatography
- Inherently and absolutely quantitative with virtually no discrimination on mass or polarity

This technology can be used for both direct sampling and with automated sampling and sample preparation.

Current and potential applications in the life sciences include:

- Real-time monitoring of industrial biotech processes (microbial and algal industrial biotechnology)
- Monitoring of Agricultural and Environmental Biogenic emissions
- Process analysis biomass combustion and biorefinery processes
- Chemical Ecology studies
- Clinical volatile biomarker (e.g., Parkinson's disease) analysis
- Direct analysis of volatile biomarkers from breath, tissues, biofluids and stools
- Microbiome studies
- Microbial identification and microbial community analysis

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POSTER 5

Nathan Hawkins - Anatune Ltd

Selected Ion Flow Tube Mass Spectrometry (SIFT-MS) - A disruptive Analytical Platform for Agriscience based on Physical Chemistry Processes

Abstract: "Selected Ion Tube Flow-Mass Spectrometry (SIFT-MS) is the latest commercially available type of Mass Spectrometer.

As an analytical platform based on physical chemistry (ion-molecule reactions based on reaction rate kinetics), it has unique characteristics in the field of mass spectrometry:

- Inherently Quantitative
- Wide linear dynamic range
- High analytical selectivity and specificity
- Low detection limits – routinely single-digit ppt(v)
- Direct sampling results in non-selective sampling
- Unsurpassed breadth of analyte chemistries (polarity and ability to analyse inorganic and organic species)
- Ability to speciate isobaric and isomeric species without chromatographic separation

The wide range of applications of SIFT-MS in the agricultural sciences will be discussed, including applications in plant biotechnology, chemical ecology, plant pathology, natural products chemistry, soil science, bio-agrochemicals, agrochemical off-drift, real-time chemical reaction monitoring, nutrient (C, N, O, S) assimilation and volatile disease biomarker validation and verification.

POSTER 6

Emily Flashman - University of Oxford

Plant Cysteine Oxidases: A Molecular View of Their Role in Plant Oxygen Sensing

Plant responses to flooding are driven by Group VII Ethylene Response Factors (ERF-VIIs) which promote transcription of genes enabling escape or quiescence flood survival strategies. ERF-VIIs are stable in the low oxygen (hypoxic) conditions experienced during flooding, but are destabilised in normoxia through the action of Plant Cysteine Oxidase (PCO) enzymes. These oxygen-sensors catalyse the oxidation of the N-terminal cysteine of the ERF-VIIs to cysteine sulfinic acid, the first step in the Cys/Arg branch of the N-end rule pathway which targets proteins for degradation. The PCOs are therefore a key step in this signalling pathway, connecting environmental stimulus with cellular and physiological response.

Manipulating the PCOs to ablate or reduce their activity may be an effective mechanism to achieve improved flood tolerance, however approaching this in a rational and targeted manner requires a detailed molecular understanding of their function. We report structural and kinetic studies of Arabidopsis PCOs which support their proposed role as plant oxygen sensors, suggest differences in substrate selectivity amongst individual PCO isoforms and which identify key active site residues, mutation of which impacts PCO activity. Collectively, this work will help direct strategies for effective modulation of PCO activity and contribute to efforts to improve plant flood tolerance.

POSTER 7

Deepti Angra, - University of Reading

Mapping of gene for synthesis of antinutritional compounds in Vicia faba using High Density Linkage Map

Abstract: Faba bean is an important legume because of the high nutritional value of its seeds, and high yield for hundreds of inhabitants in North Africa. However, it suffers from accumulation of antinutritional compounds vicine and convicine which results in development of serious haemolytic disorders in individuals. With NGS opening the door to transcriptome sequencing in this research we performed a study to discover genome wide SNPs and make them available in a convenient high throughput genotyping assay for faba bean germplasm characterization, genetic mapping and breeding applications. The high density genetic map has been generated and we are in the process of mapping the gene and developing an assay for identifying backgrounds which are low in Vicine convicine.

POSTER 8

Vicky Tagkouli - University of Reading

Simulating breeding scenarios for faba beans

Abstract: "Agroclimatic conditions in Ireland, in particular the mild winter conditions, permit high yield potential over a wide range of sowing dates in faba bean production. Despite the importance of the crop, there is neither a specifically designed breeding scheme for Irish conditions nor a breeding simulation available for that crop worldwide, able to predict relative efficiency of different selection schemes. As faba bean is a partially allogamous crop, bee pollination increases and improves seed set by transferring cross-pollen. Recurrent selection is an easily implemented breeding scheme reported to be very effective for increasing quality and agronomic traits. Here, we set up a diverse, recombining foundation population of faba bean varieties combining yield, quality and disease resistance characteristics and force a high outcrossing rate by manual creation of F1 hybrids and then using captive bumblebees during flowering to maintain a high outcrossing rate in all subsequent generations. Our selection cycles are following results of a breeding simulation software package called AlphaSim which is built in R which enables breeders to design various breeding schemes (animal and plant breeding) by simulating multiple aspects of a breeding program such as population size, selection intensity, heritability, number of QTLs, trait architecture, outcrossing rates and more.This project aims to develop a faba bean ideotype for optimal yield in autumn-sown Irish growing conditions, combining high yield performance with improved disease resistance. At the same time we explore different breeding schemes for faba beans and an opportunity for more efficient and less costly selection cycles while comparing simulated results to empirical outcomes in a prototype recurrent selection scheme.