4th INTERNATIONAL SINGAPORE LIPID SYMPOSIUM

abstracts

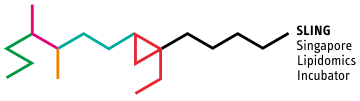
NUS National University of Singapore

4th International Singapore Lipid Symposium
13–16 March 2012

Organized by:

4th International Singapore Lipid Symposium 13–16 March 2012

Venue:	Centre for Life Sciences, National University of Singapore
Organiser:	Markus R. Wenk, National University of Singapore, Life Sciences Institute
Website:	www.lipidprofiles.com



Contents

A very warm welcome to the 4th International Singapore Lipid Symposium (ISLS).

We already mark the 4th occurrence of this biennial event which started in 2006. The founding principle of bringing together investigators with highly complementary interests is still very evident this year. ISLS is now established as a key event in the field and Asia Pacific region, and has gained global recognition as the place to keep up to date with lipid and lipidomic research.

We have adjusted the format to fit the growing needs and interests in several ways. First, we have expanded the program to four full days to offer more comprehensive coverage of topics and contents. We are proud to have an impressive list of 44 contributors which is almost double the number in 2006. A new addition are selected short talks from aspiring junior scientists in the field. This is an important contribution towards research and leadership education in a growing field. Emphasis has been shifted to highlight novel enabling technologies and examples of applications in two broad areas. Plant and environmental sciences are now complementing the original focus in biomedicine.

A second notable contribution to highlight is community building within Asia Pacific aligned with ongoing efforts of networks beyond this region. Speakers from Asia Pacific are more prominently represented than before. You will enjoy presentations from major initiatives in programmatic lipid research as well as individual laboratories with unique capabilities. In fact, times might have matured to organize concerted efforts in lipid and lipidomic research globally in the form of an organization dedicated to this field. Our colleagues in genomics and proteomics have already established Human Genome and Human Proteome organizations, 'HUGO' and 'HUPO', respectively, but we yet have to witness corresponding developments in our field. We hope that this event will contribute to these efforts. As a primer, we have decided to include three community building workshops on topics which are of current general interest in the emerging field of lipidomics. This will not only raise general awareness but will also help with integration into complementary approaches mentioned above.

I would like to express my sincere thanks to those involved in organizing the 4th ISLS. Foremost, my own research group who has been spending long extra hours in planning fine details. As part of our transformation to a strategic NUS program in lipidomics, the Singapore Lipidomics Incubator SLING, we continue to enjoy the support from the Life Sciences Institute (LSI) that is hosting this event for the 3rd time at the Centre for Life Sciences (CeLS). Many thanks also go to the Department of Biochemistry and the Department of Biological Sciences, our academic anchors at the National University of Singapore. Finally, this event would not be possible without the generous support of our partners and loyal sponsors that you find acknowledged in your handouts.

I wish you all a memorable and inspiring time at the 4th ISLS and a pleasant stay in Singapore during your visit.

Markus R. Wenk

National University of Singapore
Department of Biochemistry
Department of Biological Sciences

Organiser

Tuesday March 13 2012

Workshop 1 **"Lipid metabolism and homeostasis – case studies"** ▶ p. 13

10:00 – 12:00	Song Baoliang **Cholesterol metabolism: biosynthesis and absorption**
	Scott Summers **Ceramides as modulators of cellular and whole-body metabolism**
12:00 – 13:00	*Lunch*

Workshop 2 **"Global standards for mass spectrometry-based lipidomics"** ▶ p. 14

13:00 – 15:00	Harald Köfeler **Development of a standardized shorthand lipid nomenclature**
	Dominik Schwudke **In which way should we address lipidomic data for bioinformatic analysis? Lessons from the *Drosophila* lipidome**
	Guanghou Shui **The challenge of moving from comparative lipidomics to quantitative lipidomics**
	Todd Mitchell **Structural characterization of molecular lipids: Where do we stand?**
15:00 – 15:30	*Break*

Workshop 3 **"Curating lipidomic information"** ▶ p. 15

15:30 – 17:30	Ed Dennis **Curation challenges and solutions from the LIPID MAPS experience**
	Ioannis Xenarios **Biocuration from proteins to lipids**
	Andrej Shevchenko **Practical ways to visualize and rationalize lipidomics data**

19:00 *Reception at conference hotel (by invitation)*

Wednesday March 14 2012

	08:00	*Registration*	
	09:00	Opening remarks Prof Barry Halliwell, Deputy President NUS (Research&Technology)	
Morning session		Chair: Paul Matsudaira (NUS, Department of Biological Sciences)	
	09:20	Ed Dennis (University of California, San Diego) **Integration of genomics, proteomics, & metabolomics, finally! Lipid maps**	▶ p. 17
	10:00	Rob Parton (University of Queensland) **High resolution localization of lipids: developments and applications**	▶ p. 18
	10:40	*break*	
	11:10	Xun Huang (Chinese Academy of Sciences, Beijing) **Using *Drosophil*a and *C. elegans* models to study lipid storage**	▶ p. 19
	11:50	Andreas Zumbusch (University of Konstanz) **Coherent anti-stokes Raman scattering microscopy of intracellular dynamics**	▶ p. 20
	12:30	*lunch*	
Afternoon session		Chair: Greg Tucker-Kellogg (NUS, Department of Biological Sciences)	
	13:30	Peter Meikle (Baker IDI Melbourne) **Plasma lipid profiling in type 2 diabetes and cardiovascular disease**	▶ p. 21
	14:10	Andrej Shevchenko (MPI Dresden) **High resolution mass spectrometry for quantifying known and discovering new lipid molecules**	▶ p. 22
	14:50	Joanne Yew (Temasek Life Sciences Laboratory) **Analysis of lipid pheromones from insects using mass spectrometry**	▶ p. 23
	15:30	*break*	
	16:00	Yuki Nakamura (Academia Sinica) **Lipid metabolic switching to alter lipid levels in algae and plants**	▶ p. 24
	16:20	Brendan Prideaux (Novartis) **MALDI-MSI and LESA-MS for localization and identification of lipids in biological tissue**	▶ p. 25
	16:40	Christian Eggeling (MPI Göttingen) **Studying lipid-protein interactions in the plasma membrane of living cells with super-resolution STED microscopy**	▶ p. 26
	17:00	Guillaume Thibault (Temasek Life Sciences Laboratory) **The unfolded protein response buffers the lethal effects of lipid diequilibrium**	▶ p. 27
	17:30	*posters and reception*	
	19:30	*bus to hotel*	

Thursday March 15 2012

Morning session		Chair: Rachel Kraut (Nanyang Technological University)	
	09:00	Patricia Bassereau (Institut Curie, Paris) **What can we learn on lipid trafficking with model membrane systems?**	▶ p. 28
	09:40	Herman Overkleeft (Leiden University) **Chemical biology of glycolipid metabolism in relation to lysosomal storage disorders**	▶ p. 29
	10:20	*break*	
	10:50	Toon de Kroon (Utrecht University) **The yeast acyltransferase Sct1p regulates fatty acid desaturation by competing with the desaturase Ole1p**	▶ p. 30
	11:30	Anne-Claude Gavin (EMBL Heidelberg) **title TBA**	▶ p. 31
	12:10	Markus R. Wenk (NUS, Department of Biochemistry) **SLING, the Singapore Lipidomics Incubator**	▶ p. 32
	12:50	AB SCIEX Lunchtime invited speaker Todd Mitchell (University of Wollongong) **xxBalls, brains, LESA™ and other 5500™ adventures**	▶ p. 33
	12:50	*lunch and posters*	
Afternoon session		Chair: Scott Summers (Duke-NUS)	
	14:00	Takao Shimizu (The University of Tokyo) **Target lipidomics reveal critical molecules involved in disease onset and progression**	▶ p. 34
	14:40	Gabriele Kastenmüller (Helmholtz Zentrum München) **title TBA**	▶ p. 35
	15:20	Benhur Lee (University of California, Los Angeles) **Mechanistic basis of broad-spectrum antivirals that target the physicochemical properties of viral lipid membranes to prevent virus-cell fusion and entry**	▶ p. 36
	16:00	*break*	
	16:30	Chakravarty BN Marella (National Centre for Biological Sciences) **BLAST inspired global analysis tool box for lipidomes**	▶ p. 37
	16:50	Adam Orłowski (Tampere University of Technology) **Role of membrane cholesterol in hydrophobic matching and the resulting redistribution of proteins and lipids**	▶ p. 38
	17:10	Ajay K. Mahalka (Aalto University) **Protein-phospholipid interactions: from biophysics to therapeutics**	▶ p. 39
	17:30	Lok Hang Mak (Imperial College London) **A small molecule mimicking a phosphatidylinositol (4,5)-bisphosphate binding pleckstrin homology domain**	▶ p. 40
	18:00	*bus to conference dinner*	
	18:30	*conference dinner*	
	21:00	*bus to hotel*	

Friday March 16 2012

Morning session		Chair: Thorsten Wohland (NUS, Department of Chemistry)	
	09:00	Xu Chenqi (Chinese Academy of Sciences) **The regulation of immunoreceptor activation by phospholipid**	p. 41
	09:40	Chng Shu Sin (NUS, Department of Chemistry) **Characterization of the two-protein complex that establishes lipid asymmetry at the outer membrane of Gram-negative bacteria**	p. 42
	10:20	*break*	
	10:50	Igor Butovich (UT Southwestern) **Qualitative and quantitative lipidomic assessment of human meibomian gland secretions**	p. 43
	11:30	John Harwood (Cardiff University) **Algal lipids: topical and important**	p. 44
	12:10	Ivo Feussner (University of Göttingen) **The alphabet of galactolipids in *Arabidopsis thaliana***	p. 45
	12:50	*lunch and posters*	
Afternoon session		Chair: Markus R. Wenk (NUS, Department of Biochemistry)	
	14:00	Chye Mee-Len (The University of Hong Kong) **Acyl-CoA-binding proteins from arabidopsis and rice**	p. 46
	14:40	Neil Clarke (Genome Institute of Singapore) **Lipid metabolism and its gene regulation in model and non-model algae**	p. 47
	15:20	Chew Fook Tim (NUS, Department of Biological Sciences) **Genetic strategies for oil yield improvement in oil crops and lipid productivity in algae**	p. 48
	16:00	*break*	
	16:30	Giovanni D'Angelo (Institute of Protein Biochemistry, Naples) **Molecular mechanism of metabolic branching in the synthesis of glycosphingolipid**	p. 49
	16:50	Mathieu Blanc (University of Edinburgh) **25-hydroxycholesterol is directly coupled to the interferon response and has antiviral activities**	p. 50
	17:10	Guan XueLi (Swiss TPH) **Lipidomics of host-pathogen interactions: human macrophage as a cellular system to study functional implications of lipid metabolism during infection**	p. 51
	17:30	Thusitha Rupasinghe (The University of Melbourne) **LCMS lipid profiling of intra-erythrocyte stages and intracellular organelles of *Plasmodium falciparum***	p. 52
	17:50	*closing remarks*	
	18:30	*bus to hotel*	

Bassereau, Patricia
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Blanc, Mathieu
University of Edinburg

Butovich, Igor
UT Southwestern, Dallas

Chew, Fook Tim
National University of Singapore

Chng, Shu Sin
National University of Singapore

Chye, Mee-Len
The University of Hong Kong

Clarke, Neil
Genome Institute of Singapore

D'Angelo, Giovanni
Institute of Protein Biochemistry, Naples

De Kroon, Toon
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Gavin, Anne-Claude
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Harwood, John
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Kastenmüller, Gabriele
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Köfeler, Harald
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Lee, Benhur
University of California, Los Angeles

Lok, Hang Mak
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Mahalka, Ajay K.
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Meikle, Peter
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Mitchell, Todd
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Orłowski, Adam
Tampere Technological University

Overkleeft, Herman
Leiden University

Parton, Rob
The University of Queensland, Brisbane

Prideaux, Brendan
Novartis, Basel

Rupasinghe, Thusitha
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Chinese Academy of Sciences, Beijing

Yew, Joanne,
Temasek Life Sciences Laboratory, Singapore

Zumbusch, Andreas
University of Konstanz

Workshops

4th ISLS Workshop 1

Lipid metabolism and homeostasis—case studies

Moderator: Markus R. Wenk

Part A	presentations	30+10 min Q&A each
Song Baoliang	**Cholesterol metabolism: biosynthesis and absorption**	
Scott Summers	**Ceramides as modulators of cellular and whole-body metabolism**	

Cholesterol biosynthesis and its regulations have become text book knowledge. Ceramide metabolism is rapidly following up with many examples in health and disease. Two 25 min lectures will highlight what we know today about biosynthesis, metabolism and their regulation as well as membrane trafficking in these two cases. Presentations will contain both basic principles as well as latest research results from the laboratories of the speakers as well as others in the field.

Part B	panel discussion	40 min

Panel members Song Baoliang and Scott Summers

A general discussion to promote brain storming on topics of general interest such as:

- How will our knowledge of sterol and ceramide metabolism guide research for other lipids?
- Which similarities and differences should be highlighted?
- Total cholesterol is clinically used but how about improved future lipid markers for health and disease?
- How can we use these advances to control lipid levels and metabolic diseases?

4th ISLS Workshop 2

Global standards for mass spectrometry-based lipidomics

Moderator: Markus R. Wenk

Part A	presentations	15 + 5 min Q&A each
Harald Köfeler	**Development of a standardized shorthand lipid nomenclature**	
Dominik Schwudke	**In which way should we address lipidomic data for bioinformatic analysis? Lessons from the *Drosophila* lipidome**	
Shui Guanghou	**The challenge of moving from comparative lipidomics to quantitative lipidomics**	
Todd Mitchell	**Structural characterization of molecular lipids: Where do we stand?**	

Senior scientists involved in method development as well as routine operations of international initiatives in mass spectrometry-based lipidomics will present their experiences.

Part B	panel discussion	40min

Panel members Harald Köfeler, Dominik Schwudke, Shui Guanghou, Todd Mitchell

Key questions to be discussed:

- Diversification and specialization of experimental methods?
- Shotgun *vs*. pre-separation approaches?
- Calibration mixtures for validation of results between different sites and analytical conditions?
- Future lipid standards
- Lipid nomenclature

4th ISLS Workshop 3

Curating lipidomic information

Moderator: Markus R. Wenk

Part A	presentations	20 + 10 min Q&A each
Ed Dennis	**Curation challenges and solutions from the LIPID MAPS experience**	
Ioannis Xenarios	**Biocuration from proteins to lipids**	
Andrej Shevchenko	**Practical ways to visualize and rationalize lipidomics data**	

Mass spectrometry-based lipidomics has led to the generation of detailed catalogues of biochemical data. LIPID MAPS has been the leading initiative to establish a gateway for lipidomics. Maintenance of curation of such large reference and knowledge platforms will require not only broad interest from the user community, but will also benefit from experiences gathered in genomics and proteomics.

Part B	panel discussion	30min

Panel members Ed Dennis, Ioannis Xenarios and Andrej Shevchenko

Key questions to be discussed:

- In what ways can the lipid community learn from reference bases in genomics and proteomics?
- What should ultimately be included in a comprehensive lipidomic database?

Abstracts of oral presentations

Integration of genomics, proteomics, & metabolomics, finally! Lipid maps

Edward A. Dennis

University of California, San Diego, USA

The omics evolution began at the end of the 20th century with the cloning of the human genome. The 21st century has already seen the development of comprehensive proteomics analyses, but the emerging evolution is to metabolomics, the definition of which is the identification and quantitation of all of the molecular constituents of the cell including its nucleic acids, amino acids, sugars, and fats. But by far, the largest number of distinct molecular species in cellular metabolism lies in the fats (or lipids) where tens of thousands of distinct molecular species exist in cells and tissues.[1] We have now applied novel liquid chromatographic-mass spectrometric based lipidomics techniques termed "class"[2] generally in the context of an overall omics analysis of immunologically-activated macrophages integrating transcriptomics, proteomics, and metabolomics of lipid metabolites.[3] As part of the lipid maps Consortium [see, lipid maps/Nature Lipidomics Gateway www.lipidmaps.org], our laboratory has developed a robust and comprehensive approach to the lipidomic analysis of hundreds of fatty acids, acylethanolamines and inflammatory eicosanoids, including their numerous metabolites arising from an array of cyclooxygenases, lipoxygenases, cytochrome P450s and non-enzymatic oxidation producing isoprostanes, as well as combinations thereof.[4] We will discuss the application of lipidomic analysis to characterize cellular lipid signaling of Toll-like (TLR) and purinergic receptors and their "synergy" in endotoxin stimulated macrophages as models for inflammation and infection.[5] New results comparing various primary macrophages and analysis of the fluxes of metabolites will be presented. Also lipidomic analysis of cells supplemented with small amounts of the omega-3 fatty acids eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA) provides information on the overall effects of EPA and DHA on the inflammatory eicosadome. Human plasma has also been profiled to quantify almost six hundred distinct lipid molecular species present across all mammalian lipid categories[6] and the implications for the future of clinical medicine and the understanding of the mechanisms of disease will be discussed. [7]

[1] Dennis, *Proc. Natl. Acad. Sci. USA.*, *106:*2089–2090 (2009)

[2] Harkewicz & Dennis, *Ann. Rev. Biochem.*, 80:301–25 (2011)

[3] Dennis *et al.*, *J. Biol. Chem*, 285:39976–85 (2010)

[4] Buczynski *et al.*, *J. Lipid Res. 50:*1015–1038 (2009)

[5] Buczynski *et al.*, *J. Biol. Chem., 282*:22834–22847 (2007)

[6] Quehenberger *et al.*, *J. Lipid Res.*, 51:3299–3305 (2010)

[7] Quehenberger & Dennis, *New England Journal of Medicine*, 365:1812 (2011)

Supported by LIPID MAPS Glue Grant U54 GM069338, R01 GM020501 and R01 GM064611.

High resolution localization of lipids: developments and applications

Robert G. Parton

Institute for Molecular Bioscience and Centre for Microscopy and Microanalysis, University of Queensland.

Lipids are the major structural components of cellular membranes as well as crucial signalling molecules. The multiple roles of lipids are dependent on specific localization to cellular organelles and to domains within those membrane systems. The importance of understanding lipid regulation is emphasized by the vast number of disease conditions involving lipid dysregulation, including diabetes, atherosclerosis, and Alzheimer's disease. While the post-genomic era of biology has brought advances in understanding the role of cellular proteins, the study of lipids is still technically challenging. Lipid localization by imunoelectron microscopy is particularly problematic owing to the technical difficulties associated with retaining lipids in the cells during the labeling procedure and the lack of specific markers for lipids. Methods to localize lipids at high resolution by electron microscopy would represent a major contribution to membrane biology. I will describe the development of methods to localize lipids such as phosphatidylserine, phosphatidylethanolamine, and ceramide at the electron microscopic level with particular reference to our work on caveolae. These methods which involve high pressure freezing, rapid freeze substitution, and embedding in resin at low temperature should be applicable to the study of many different lipids in cells, tissues and whole organisms and compatible with colabelling of endogenous proteins.

Using *Drosophila* and *C. elegans* models to study lipid storage

Xun Huang

Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing, China

Within the cells, lipid homeostasis is achieved through several lipid metabolic processes including lipid biosynthesis, lipid utilization, lipid storage, and lipid trafficking. Adipose tissues are the mainly lipid storage sites. Under certain conditions, excess lipid accumulation could lead to many disease states including obesity and diabetes. In addition, ectopic lipid storage in non-adipose tissue results in a lipotoxicity effect in many conditions such as insulin resistant in diabetes and heart failure. We mainly use *Drosophila* and *C. elegans* to study the regulation of lipid metabolism in adipose tissue, fat body, and non-adipose tissues. We generated *Drosophila* model to study the underlying mechanism of the most severe form of human lipodystrophy, Berardinelli-Seip Congenital Lipodystrophy 2, which is caused by mutations in the BSCL*2/Seipin* gene. In addition to reduced lipid storage in the fat body, *dSeipin* mutant flies accumulate ectopic lipid droplets in the salivary gland, a non-adipose tissue. Our data suggest that dSeipin may participate in phosphatidic acid metabolism and subsequently down-regulate lipogenesis to prevent ectopic lipid droplet formation in a tissue-autonomous fashion. To identify more factors involved in lipid storage regulation, several genetic screens were conducted in both flies and worms. Recent progress will be reported in the meeting.

Coherent anti-stokes Raman scattering microscopy of intracellular dynamics

Andreas Zumbusch

Department Chemie, Universität Konstanz, Konstanz, Germany

During the last decade, ultrasensitive microscopy has become one of the most important tools in biophysics. Most prominent among the various techniques is fluorescence microscopy. It is a very widespread technique with which sensitivities down to the single molecule detection can be accomplished. An important recent development is its application in the tracking of dynamic processes in biological samples, *e. g.* intracellular transport of organelles or nanoscopic particles. The necessity to label the samples and photobleaching, however, limit the scope of fluorescence microscopy in this field.

CARS microscopy as a label free approach[1] is an attractive alternative. While it does not feature the high sensitivity of fluorescence microscopy, its contrast generation on vibrational molecular spectra circumvents both the labeling and the photobleaching problem. After a comparison of CARS microscopy to fluorescence microscopy as tools for following intracellular dynamics, this talk will present the current state-of-the art of experimental equipment for CARS microscopy.[2,3] Applications to the tracking of intracellular transport of DNA and of lipid metabolism and lipid droplet trafficking will exemplify the high potential of CARS microscopy.[4]

[1] M. Müller, A. Zumbusch, *Chem. Phys. Chem.*, 8:2156–2169 (2007)

[2] G. Krauss *et al.*, *Opt. Lett.*, 34:2847–2849 (2009)

[3] R. Selm *et al.*, *Opt. Lett.*, 35:3282–3284 (2010)

[4] C. Jüngst *et al.*, *J. Biophot.*, 4:435–441 (2011)

Plasma lipid profiling in type 2 diabetes and cardiovascular disease

Peter J. Meikle

Baker IDI Heart and Diabetes Institute, Melbourne, Australia

The metabolic syndrome incorporating obesity, hypertension, dyslipidemia and elevated plasma glucose has reached epidemic proportions in many countries leading to an increased prevalence of type 2 diabetes (T2D) and cardiovascular disease (CVD). Dyslipidemia, as assessed by standard measures (raised plasma triglycerides and LDL-cholesterol, and decreased HDL-cholesterol) is an independent risk factor for T2D and CVD. However, current risk prediction algorithms have limited accuracy. Further to this, the mechanistic links between dyslipidemia, T2D and CVD are complex and not well understood. Lipidomics presents a new set of tools to address these issues.

We have developed a targeted lipidomics platform using liquid chromatography electrospray ionization-tandem mass spectrometry to profile 300–400 lipids from 10 μL plasma. We have applied this technology to multiple clinical and population based cohorts to define the plasma lipid profiles associated with T2D and CVD and evaluate the potential application of these profiles to assess disease risk and inform on disease biology.

Binary logistic regression analysis adjusting for covariates (age, sex, systolic blood pressure and obesity) identified multiple lipid species that were significantly associated with T2D or CVD. Many of these lipids also displayed an association with disease severity suggesting that they are altered prior to the onset of acute stage disease. Multivariate analysis incorporating unsupervised feature correlation minimization and relief feature selection was employed to create and test multivariate classification models incorporating different numbers of lipids and other risk factors. Relatively few lipids (5–30) were required to achieve maximum classification accuracy. Models based on lipids generally performed better than models based solely on traditional risk factors. Validation of these findings on independent cohorts and application to prospective studies is providing additional evidence of these findings.

Plasma lipid profiling can provide insight into disease pathogenesis and may contribute to a new approach to risk stratification for T2D and CVD.

High resolution mass spectrometry for quantifying known and discovering new lipid molecules

Andrej Shevchenko

Max Planck Institute, Dresden, Germany

High mass resolution of modern mass spectrometers is changing the paradigm of lipidomic analyses. By placing greater emphasis on the value of accurately determined intact masses, it paves the way for comprehensive, yet very rapid quantitative profiling of major constituents of complex lipidomes. It is becoming possible to systematically assess the lipidome response towards global challenges (food, environment, organism development), or evaluate "ripple" effects of individual mutations that are spanning through the entire lipidome. Intriguingly, high mass resolution MS and MS/MS also facilitate systematic screening for yet unknown lipid classes or individual molecules. The talk will address practical aspects of shotgun and LC-MS lipidomic analyses on LTQ Orbitrap and Q Exactive platforms; the impact of instrument-dependent features and available software solutions.

Analysis of lipid pheromones from insects using mass spectrometry

Joanne Y. Yew

Temasek Life Sciences Laboratory and Department of Biological Sciences, National University of Singapore, Singapore

Many animals communicate through pheromones, chemical signals that can mediate behaviors as diverse as kinship, aggression, and mate choice. Insects, in particular, express an impressive complexity of pheromone signals on the external cuticle. The majority of insect pheromones are lipophilic hydrocarbons and fatty acids. Structural features such as the number and position of double bonds, functional groups, and stereochemistry contribute to signal specificity. By eavesdropping on this chemical language, we can develop strategies to control populations of insect pests like mosquitoes by manipulating their reproductive behavior. Moreover, the study of pheromone diversity, biosynthesis, and perception provides a means to address fundamental questions in biology such as the neural substrates of learning and memory, the role of sexual selection in the evolution of new species, and the biochemistry of lipid production.

Mass spectrometry (MS) technology can be integrated with behavioral studies in order to capture a real-time biological picture of pheromone expression. I will discuss the application of laser desorption/ ionization (LDI) MS and ambient MS techniques towards 1) the discovery of novel pheromones and 2) the rapid pheromone profiling of single intact insects. Recent findings include discovery of *Drosophila* sex pheromones, identification of novel components of a pheromone biosynthetic pathway, and validation of MS pheromone profiling as a rapid means of species classification.

Lipid metabolic switching to alter lipid levels in algae and plants

Yuki Nakamura[1,2]

[1] *Institute of Plant and Microbial Biology, Academia Sinica, Taipei, Taiwan,* [2] *Japan Science and Technology Agency, PRESTO, Saitama, Japan*

Changing lipid levels in vivo is not easily achieved due to the tight regulation of lipid metabolism. In transgenic approach, constitutive over-expression or suppression of the lipid biosynthetic gene may cause a lethal effect because of its pivotal role, or cause no effect because some alternative pathways can compensate for the altered lipid composition. We are creating a new system to control lipid levels in algae and plants. This system, termed as "lipid metabolic switching system", is based on an inducible promoter followed by a gene coding for a lipid biosynthetic enzyme or an artificially designed microRNA which targets mRNA of specific lipid biosynthetic gene for degradation. Upon chemical or environmental trigger to which the inducible promoter responds, the promoter switch is turned on to over-express encoded gene. Once the trigger is removed, the metabolic flow switches back to the original state.

This system has employed to study on physiological function of cellular lipid levels in plants and is now applied to engineer lipid metabolism in algae for bioenergy creation.

MALDI-MSI and LESA-MS for localization and identification of lipids in biological tissue

Brendan Prideaux[1], Dieter Staab[1], Veronique Dartois[2], Laura Via[3], Danielle Weiner[3], Clifton E Barry[3] and Markus Stoeckli[1]

[1] *Novartis Institutes for BioMedical Research, Basel, Switzerland,* [2] *Novartis Institute for Tropical Diseases, Singapore,* [3] *National Institutes of Health, Bethesda, USA*

Since its introduction into the field of bioanalysis, MALDI Mass Spectrometry Imaging (MALDI-MSI) has been increasingly utilized to visualize lipid localization in biological tissues. Liquid Extraction Surface Analysis (LESA) is a recently developed method in which micro liquid extraction is performed directly on a tissue surface and the resulting extract analyzed using electrospray ionization MS. LESA when coupled with Orbitrap MS enables the rapid identification of non-isobaric analytes without the need for MS/MS due to the high mass resolving power and accuracy offered. As such, it can be used as a complementary method to MSI for the identification of multiple lipids in a single experiment.

The application of both technologies to tuberculosis (TB) research will be presented. TB is a common and frequently deadly disease caused by *Mycobacterium tuberculosis* (MTB). Of particular interest in understanding both TB pathology and potential drug treatment are the characteristic granulomatous lesions that form in the lungs of infected patients and the role both host and mycobacterial lipids play in their development.

In these experiments we have utilized a rabbit model infected with *Mycobacterium tuberculosis* (HN878 strain) that has been shown to develop advanced necrotic and caseous lesions mimicking human infection. Biopsies containing areas of normal lung and granuloma were taken following necropsy and 14 μm sections were prepared for MS Imaging. Using Q-ToF MS imaging, lipids were identified specific to normal lung tissue, granuloma tissue containing viable macrophages, necrotic and caseous granuloma areas. Identification of the lipids was confirmed by MS/MS. The spatial limitations of the LESA measurements were defined by the droplet size (1–1.5 mm diameter) and were insufficient to resolve the viable granuloma from the necrotic tissue. However, >50 lipids were identified using the high mass resolution capabilities of the Orbitrap.

Novel lipid MS neutral loss and precursor ion scan imaging methods utilizing triple quadrupole MS instrumentation will be presented and demonstrated with preliminary data from whole-body rat tissue sections.

Studying lipid-protein interactions in the plasma membrane of living cells with super-resolution STED microscopy

Christian Eggeling, Alf Honigmann, Veronika Mueller and Stefan W. Hell

Department of Nanobiophotonics, Max Planck Institute for Biophysical Chemistry, Göttingen, Germany

Interactions of lipids and proteins in the plasma membrane of living cells such as the integration into lipid nanodomains are considered to play a functional part in a whole range of cellular processes. Unfortunately, the direct and non-invasive observation of these interactions in living cells is impeded by the resolution limit of >200 nm of a conventional far-field optical microscope. We report the detection of membrane heterogeneities in nanosized areas in the plasma membrane of living cells using the superior spatial resolution of stimulated emission depletion (STED) far-field nanoscopy. By combining a (tunable) resolution of down to 30 nm with tools such as fluorescence correlation spectroscopy (FCS), we obtain new details of molecular membrane dynamics. Our first studies revealed how sphingolipids or other proteins are transiently (~10 ms) trapped on the nanoscale in molecular complexes, while others diffuse freely or show a kind of hopping diffusion[1–3]. Distinct differences especially with respect to the dependence on cholesterol and the underlying cytoskeleton showed up[4]. We will revisit these results and – besides novel applications of this novel STED-FCS technique – show a comparison of the plasma membrane data to STED experiments on model membranes, which highlight potential influences of the fluorescent tag but also give new insights into the existence of lipid nanodomains. Our observations shed new light on the role of lipid-protein interactions and nanodomains for membrane bioactivity.

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The unfolded protein response buffers the lethal effects of lipid disequilibrium

Guillaume Thibault[1], Guanghou Shui[2], Markus R. Wenk[2,3] and Davis Ng[1]

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Hundreds of distinct lipids in varying concentrations assemble to form biological membranes. In eukaryotes, lipid compositions can differ widely among organelles and even between leaflets of a membrane bilayer. Accordingly, lipid homeostasis is critical as disequilibrium can lead to disease. The mechanisms that monitor and maintain lipid homeostasis, however, remain poorly understood. Here, we show that the unfolded protein response (UPR) stress pathway plays a major role in maintaining the functional integrity of membranes. Upon phospholipid imbalance, the UPR detects the stress and activates a compensatory program that maintains cell viability. Unexpectedly, lipidomic profiling reveals that the UPR need not restore lipid composition to alleviate stress. Instead, it can maintain the organization of organelles and membrane protein function in the new state. The remarkable robustness of the UPR in this role is illustrated by its ability to maintain cell growth even after depleting its most abundant phospholipid from membranes.

What can we learn on lipid trafficking with model membrane systems?

Patricia Bassereau

PhysicoChimie Curie, Institut Curie, Paris, France

Membrane deformation mechanisms of cell membranes by proteins but also lipid sorting mechanisms are actively studied in the cell biology context. However, there is a long history of membrane physics, which can help to address this question. For more than 30 years, physicists have worked on developing theories and in vitro systems in order to model cell membranes. Systems with different geometries and controlled composition are available, among them Giant Unilamellar Vesicles (GUV), allowing for a direct comparison with theoretical models. We will see how membrane curvature and interactions between proteins deforming membranes and lipids can drive lipid sorting. In order to study the effect of membrane curvature, membrane nanotubes pulled from GUV with a controlled diameter (15–500 nm) are very convenient model systems. We will show that curvature-induced lipid sorting only occurs if the membrane is close to a demixing point. In addition, for these compositions, lipid sorting is further amplified when even a low fraction of lipids is clustered upon cholera toxin binding suggesting that lipid-clustering proteins may play an important role in curvature-induced sorting in biological membranes. Eventually, with another toxin, the B-subunit of Shiga toxin binding to Gb3 lipids and inducing tubular invaginations both in vivo and in vitro, we will demonstrate that the invaginations induced by STxB are enriched in sphingomyelin, and depleted from DOPC. This suggests that lipids interacting favorably with a membrane deforming protein or with its receptor can be co-sorted in curved structures, overriding the curvature-induced sorting effect.

Chemical biology of glycolipid metabolism in relation to lysosomal storage disorders

Herman Overkleeft

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Aberrations in glucosylceramide metabolism are at the basis of several human disorders, including the lysosomal storage disorder, Gaucher disease, and type 2 diabetes. Factors involved in glucosylceramide metabolism are therefore valid targets for drug development. In this lecture I will report on the progress we made in the development of deoxynojirimycin-type inhibitors selective for each of the glycoprocessing enzymes involved in glucosylceramide metabolism, namely glucosylceramide synthase (GCS), acid glucosylceramidase (GBA1) and neutral glucosylceramidase (GBA2). Secondly, I will discuss our progress in designing activity-based probes for the profiling of retaining beta-glucosidases, including those involved in glucosylceramide metabolism. In our studies we compared 2-deoxy-2-fluoroglycosides and cyclitol epoxides and we evaluated the merits of direct and two-set bioorthogonal labeling strategies to arrive at a highly sensitive acid glucosylceramidase probe with which this activity can be monitored in living cells. I will present our results in the use of this probe in monitoring enzyme activity in the context of Gaucher disease and in the presence of enzyme inhibitors/activity-based probes. I will discuss our current (unpublished) progress in the development of activity-based probes targeting a broader range of retaining glycosidases. Thirdly, I will discuss our (unpublished) progress in the development and application of stable isotope-enriched glycolipids as markers to monitor glycoshingolipid metabolism in health and disease.

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The yeast acyltransferase Sct1p regulates fatty acid desaturation by competing with the desaturase Ole1p

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Membrane fluidity is essential for maintaining the membrane barrier and for optimal functioning of membrane proteins, and is to large extent determined by the proportions of saturated and unsaturated acyl chains of the membrane lipids. *S. cerevisiae* readily adapts its acyl chain saturation when shifted to different temperatures to maintain membrane fluidity.

Tuning of the expression of the yeast's single fatty acid desaturase Ole1p was until now the only mechanism known by which acyl chain desaturation is adapted. The expression of the OLE*1* gene is regulated by the carbon source, the presence of fatty acids in the growth medium and oxygen levels[1].

In research on acyl chain remodeling of the major membrane lipid phosphatidylcholine (PC), we obtained clues for a novel mechanism regulating fatty acid desaturation that involves the glycerol-3-phosphate acyltransferase Sct1p/Gat2p and is juxtaposed to the regulation of Ole1p activity.

A screen for gene products required for PC remodeling by acyl chain exchange, revealed a role for Sct1p/Gat2p in the incorporation of C16:0 acyl chains into newly synthesized PC. The SCT*1* gene was first characterized as a multicopy suppressor of a choline transport mutant also defective in the methylation of phosphatidylethanolamine (PE)[2], hence the name Suppressor of Choline Transport 1. Later SCT*1* was found to encode the enzyme catalyzing the first committed step in glycerolipid synthesis, *i. e.* the transfer of an acyl chain from acyl-CoA to glycerol-3-phosphate yielding 1-acyl-2-lyso-*sn*-glycero-3-phosphate[3].

Deletion of SCT*1* was found to decrease the cellular content of saturated fatty acids. Overexpression of Sct1p increased the C16:0 content over 2-fold and changed the growth rate and the cellular phospholipid and neutral lipid profiles. Experiments in which Ole1p and Sct1p were co-overexpressed indicated competition between the two enzymes for C16:0-CoA.

We propose that Sct1p shields C16:0-CoA and other saturated acyl-CoAs from the introduction of a double bond by Ole1p by preferentially sequestering the acyl chains into lipids. Based on additional experimental evidence, we furthermore propose that the competition for substrate with the desaturase Ole1p is determined by the activity of Sct1p that in turn depends on the expression level and the phosphorylation state of the enzyme.

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Title TBA

Anne-Claude Gavin

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http://www.embl.de/research/units/scb/gavin/members/?s_personId=4074

SLING – the Singapore Lipidomics Incubator

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Once viewed simply as a reservoir for carbon storage, lipids are no longer cast as bystanders in the drama of biological systems. The emerging field of lipidomics is driven by technology, most notably mass spectrometry, but also by complementary approaches for the detection and characterization of lipids and their biosynthetic enzymes in living cells. The development of these integrated tools promises to greatly advance our understanding of the diverse biological roles of lipids.[1]

In this presentation, I will give an overview of SLING, the Singapore Lipidomics Incubator. Shaped from a five year competitive research program supported by the National Research Foundation and the National University of Singapore, this centre is a major global magnet for collaborating parties in lipidomics – from academia and industry – delivering new technologies and intellectual capital.

I will highlight our progress in developing new tools for the detection of lipids and lipid enzymes in procaryotic systems, where lipid chemistry, especially diverse in mycobacterial surface coats, is of relevance for interaction with the host.[2] Using liquid chromatography and electrospray ionization mass spectrometry we were able to demonstrate that C26 mycolic acids are diagnostic markers for tuberculosis case detection in humans and drug efficacy in mice.[3] Translation of such findings to other experimental readouts, such as antibody based detection, is in progress for several bacterial surface (glyco)lipids. We have also used a chemical biology approach to capture lipid enzymes with novel compounds that were designed and synthesized in our program. These substrate analogues bind lipases and related enzymes and thus allow for discovery of factors involved in lipid metabolism via proteomics.[4,5] Indeed, 'selective capture', not only of enzymes but instead of lipids will be a main aim of SLING. Development of such new workflows for integrated lipid analysis will greatly facilitate studies which aim to understand natural variation of lipids in biological systems.

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INVITED LECTURE

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AB SCIEX Lunchtime invited speaker, **Todd Mitchell**

University of Wollongong, Wollongong, Australia

http://www.uow.edu.au/health/healthsciences/staff/UOW028956.html

Target lipidomics reveal critical molecules involved in disease onset and progression

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Glycerophospholipids constitute ~1,000 different molecular species by the combination of fatty acids at sn-1, sn-2 positions, and polar head group at sn-3. Membrane dynamics and multiplicity are maintained by the concerted actions of phospholipase A1/2 (PLA1 and PLA2) and lysophospholipid acyltransferases. PLA2 is an enzyme that cleaves a sn-2 ester bond and produced polyunsaturated fatty acids, and lysophospholipids, both are precursors of multiple lipid mediators. We found a variety of abnormalities in physiology and pathology of mice deficient in cytosolic phospholipase A2a (also termed GIVA).[1] To understand the molecular mechanisms underlying these phenotypes, targeted lipidomics analyses using LC-MS focusing on membrane glycerophospholipids and downstream lipid mediators are devised.[2] Using a disease model mice and patient samples, we found that PGE2 plays critically important role in induction of allergic encephalomyelitis (EAE), while PAF (platelet-activating factor) is an exacerbating factor in the chronic phase of the disease.[3,4,5] Similarly, in both fibrosis patients and bleomycin-induced murine lung fibrosis, PGF2a is important in fibrotic lung changes.[6] These methods are useful to identify key lipid mediators in various stages of diseases thus providing novel targets to either prevent or treat patients. Related studies on lysophospholipid mediators (like LPA, lysoPS, lysoPC) and lysophospholipid acyltransferases will be discussed.[7,8]

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Title TBA

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Mechanistic basis of broad-spectrum antivirals that target the physicochemical properties of viral lipid membranes to prevent virus-cell fusion and entry

Frederic Vigant[1], Jihye Lee[2], Zeynep Akyol-Ataman[1], Tatyana Yun[3], Lukas Tanner[4], David Meriwether[6], Shui Guanghou[4], Terry Juelich[3], Dong Zhang[5], Gleyder Roman-Sosa[7], Lindsey Robinson[1], Hector Aguila-Carreno[1], Mike Wolf[6], Margaret Kielian[7], Markus R. Wenk[4], Srinivasa Reddy[6], Matthias Selke[5], Alexander Freiberg[3], Michael Jung[2] and **Benhur Lee**[1]

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LJ001, a small molecule broad-spectrum antiviral, inhibits the entry of many enveloped viruses with an IC_{50} of ffi 1 μM (Wolf *et al.*, PNAS, 2010). LJ001 intercalates into viral membranes, inhibits viral entry at a step post-binding but prior to viral-cell fusion, and exploits the physiological difference between static viral membranes and biogenic cellular membranes with reparative capacities. We now report on the molecular mechanism that results in LJ001's specific inhibition of virus-cell fusion.

LJ001 is a photosensitizer that is activated by light to generate singlet oxygen (BO_2) within the plane of membranes. Thus, LJ001's antiviral activity was light-dependent, but can also be reversed by antioxidants or BO_2 quenchers. BO_2 reacts readily with alkenes (carbon double bonds), present in unsaturated phospholipids and cholesterol in viral membranes. A panoply of assays showed unsaturated phospholipids to be the main target modified by LJ001 generated BO_2. BO_2 mediated allylic hydroxylation of unsaturated phospholipids leads to a *cis:trans* migration of the double bond and concurrent formation of a hydroxyl group in the middle of the hydrophobic lipid bilayer. Hydroxylated fatty acid species were detected in model and viral membranes treated with LJ001, but not its inactive analog, LJ025, which differed from LJ001 by a single atomic change. Cis-to-trans conversion of the double bond leads to closer packing of the phospholipid species, while hydroxylation of the acyl chain leads to clustering of the hydrophilic species in membrane microdomains. The former increases positive curvature while the latter increases membrane rigidity. A combination of these factors perturbs the balance of proper phospholipid species required for the positive to negative curvature transition that occurs during virus-cell fusion.

Based on our understanding of LJ001's mechanism of action, we designed a new class of photosensitizers to overcome LJ001's limitations for use as an *in vivo* antiviral agent. SAR analysis led to a novel class of compounds (oxazolidine-2,4-dithiones) with (1) 100-fold improved *in vitro* potency (IC_{50} 10 nM), (2) red-shifted absorption spectra and maxima (longer wavelengths have better tissue penetration), (3) increased quantum yield, which relies on physical properties such as the efficiency of singlet oxygen generation across its absorption spectrum, and (4) 10–100-fold improved bioavailability. Candidate compounds in our new series demonstrated encouraging *in vivo* activity, significantly delaying the time to death, in a murine lethal challenge model of Rift Valley Fever Virus.

BLAST inspired global analysis tool box for lipidomes

Chakravarty BN Marella and Dominik Schwudke

National Centre for Biological Sciences, Bangalore, India

Renewed interest in the biological role of lipids coupled with technological advances in mass spectrometry has led to an increase in number of published lipidomes. Studies investigating the impact of lipidome changes accompanying developmental processes and disease progression are expected to increase in coming years.

However, methods to visualize and quantify "qualitative" changes in lipidome are limited. Aim of this study is to develop informatics resources that account for chemical similarity between lipid molecules as basis for a qualitative comparison of lipidomes.

This study investigates 1) How large ensembles of lipid molecules and their chemical structure can be computationally represented 2) Which algorithms are suitable for unsupervised determination of chemical similarity between lipid molecules 3) How to visualize lipidomes consisting of hundreds of molecules?

Lipidomes can be embodied as databases of SMILES strings, which are handled as individual sequences. We performed systematic analysis of a number of string comparison algorithms (Levenshtein distance, Smith-Waterman Local Alignment, Multiple Sequence Alignment etc) for efficient and accurate measurement of chemical similarities. As results, we show that alignment based algorithms resolve minute positional changes in isomeric lipid molecules and have the potential to differentiate all molecules of a Lipidome.

As a test dataset, we generated 1185 molecules that are comprised of 21 Fatty Acids, 12 Sphingoid bases, 288 Ceramides, 288 Ceramide Phosphoethanolamines, 288 GlucosylCeramides and 288 Ceramide Phosphi-Inositols. Our approach based upon Non-Canonical SMILES representation and Multiple Sequence Alignment as chemical similarity measure, outperformed all other tested methods and an analysis on a 3.07 GHz processor would take 2.73 minutes.

Finally, we tested the practicality of our approach by performing similar analysis on the entire LipidMaps database using dimensional scaling of inter-molecular similarities. Our unsupervised informatics approach structured and separated all lipid classes according to LipidMaps classification.

We envision that this kind of informatics approach will help to understand the influence of lipid metabolism in cell differentiation, degenerative processes and usage of disease models.

Role of membrane cholesterol in hydrophobic matching and the resulting redistribution of proteins and lipids

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One of the physical mechanisms leading to lateral self-organization of cell membranes is the hydrophobic mismatch between a lipid membrane and the transmembrane part of a membrane protein. Meanwhile, cholesterol is in many ways a unique molecule with regard to its capability to promote membrane order and control the physical properties of lipids around it. In this spirit, it is tempting to consider how cholesterol could contribute to hydrophobic mismatch. The topic is particularly exciting given that there is a gradient of cholesterol along the secretory pathway, implying that the changes in membrane properties due to varying concentration of cholesterol can be an important factor for the sorting of nonmatched Golgi transmembrane proteins.

We have combined atomistic simulations with a major arsenal of experimental techniques to study the role of cholesterol in hydrophobic mismatch as well as its biological consequences. We have observed cholesterol to play a central role in controlling structural adaptations at the protein-lipid interface under mismatch. This is shown to result in a sorting potential that leads to selective segregation of proteins and lipids according to their hydrophobic length. The results allow us to provide a mechanistic framework for a better description of the organizing role of cholesterol in eukaryotic membranes.

Adam Orłowski is the recipient of one of the 4th ISLS travel awards, sponsored by the EUROCORES Programme of the European Science Foundation (www.esf.org) "EuroMEMBRANE" (Membrane Architecture and Dynamics: www.esf.org/euromembrane).

Protein-phospholipid interactions: from biophysics to therapeutics

Ajay K. Mahalka and Paavo K.J. Kinnunen

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Lipid–protein interactions play a key role in a large number of cellular processes and are controlled by the membrane associated physicochemical properties and structure of membrane lipids. Recently, several lines of evidence have merged to suggest that membranes containing negatively charged and/or oxidized phospholipid (oxPL) cause the accumulation of the cytotoxic peptides onto the membrane surface and induce their aggregation and subsequent conversion to amyloid, with intermediate cytotoxic oligomers being responsible for killing the cells and causing loss of tissue function in conditions such as Alzheimer's and Parkinson's disease, prion disease, and type 2 diabetes BKC. Interestingly, the same underlying mechanism involving lipids has been recently concluded to be responsible also for the targeting of host defence proteins[3]. We also demonstrated that 1-palmitoyl-2-(9'-oxononanoyl)-sn-glycero-3-phosphocholine (PoxnoPC), an aldehyde-derivatized oxPL, accelerates Finnish type familial gelsolin amyloidosis *in vitro*[4].

In addition to counteracting protein aggregation, the evolutionarily conserved chaperone Hsp70 (heat shock protein 70) promotes cell survival by *e. g.* inhibiting the permeabilization of lysosomal membranes[5]. Hsp70 binds to bis-monoacylglycero phosphate (BMP, also known as lysobisphosphatidic acid), an acidic phospholipid enriched in lysosomes. This interaction with BMP appears to be specific, pH-dependent and required for the activation of the lysosomal acid sphingomyelinase (aSMase) and the subsequent stabilization of lysosomes by ceramide, underlying the downstream cytoprotective effect of Hsp70, which can be blocked by an antibody against BMPF.

The mechanism underlying the activation of SMase is unclear. We have recently forwarded a novel type of functional amyloid formation in the control of phospholipase A2 (PLA2), with low activity enzyme monomers converting to highly active oligomers and subsequently to inactive amyloid, this process acting as a self-propagating on-off switch for PLA2 activity[6]. Along these lines we also showed Hsp70 to activate PLA2 *in vitro* and suggested this activation to result from Hsp70 prolonging the lifetime of the high activity oligomers, counteracting its inhibition by conversion to amyloid[7].

Our recent data also suggests that Hsp70 has specifically associates with BMP, cardiolipin, and phosphatidylserine, respectively, in lysosomes, mitochondria, and the outer plasma membrane surface of cancer cells involving pathological conditions and these interactions controlling the orientation and conformation of Hsp70 in membrane[8]. In conclusion protein-phospholipid interactions open novel venues for the development of therapeutics for amyloid, lysosomal storage disorders (Niemann-pick disease), and cancer.

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Ajay K. Mahalka is the recipient of one of the 4th ISLS travel awards, sponsored by the EUROCORES Programme of the European Science Foundation (www.esf.org) "EuroMEMBRANE" (Membrane Architecture and Dynamics: www.esf.org/euromembrane).

A small molecule mimicking a phosphatidylinositol(4,5)-bisphosphate binding pleckstrin homology domain

Lok Hang Mak[1,3], Savvas N. Georgiades[1], Evelyn Rosivatz[2], Gillian F. Whyte[1,3], Marianna Mirabelli[1], Ramon Vilar[1,3] and Rudiger Woscholski[1,3]

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Inositol phospholipids have emerged as important key players in a wide variety of cellular functions. Among the seven existing inositol phospholipids, phosphatidylinositol(4,5)-bisphosphate ($PI(4,5)P_2$) has attracted much attention in recent years due to its important role in numerous cellular signaling events and regulations, which in turn impact several human diseases. This particular lipid is recognized in the cell by specific lipid binding domains, such as the Pleckstrin-homology (PH) domain, which is also employed as a tool to monitor this important lipid. Here, we describe the synthesis and biological characterization of a small molecule that mimics the PH domain as judged by its ability to bind specifically to only $PI(4,5)P_2$ and effectively compete with the PH domain *in vitro* and in a cellular environment. The binding constant of this small molecule PH domain mimetic (PHDM) was determined to be 17.6 ± 10.1 μM, similar in potency to the PH domain. Using NIH 3T3 mouse fibroblast cells we demonstrated that this compound is cell permeable and able to modulate $PI(4,5)P_2$-dependent effects in a cellular environment such as the endocytosis of the transferrin receptor, loss of mitochondria as well as stress fiber formation. This highly $PI(4,5)P_2$-specific chemical mimetic of a PH domain, is not only a powerful research tool, but might also be a lead compound in future drug developments targeting PI(4,5)P2-dependent diseases such as Lowe syndrome.

Lok Hang Mak is the recipient of a 4th ISLS SLING travel award.

The regulation of immunoreceptor activation by phospholipid

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Plasma membrane is an intriguing asymmetrical environment providing home for ~30% proteins encoded by mammalian genome. The spatiotemporal regulation of the plasma membrane lipid components directly influences the membrane protein structure and function, which in turn affects cell signaling and correlative biological processes. In mammalian immune system, cell surface immunoreceptors play key roles in defending the body against invading pathogens by transducing extracellular ligand-binding signals to intracellular signals to make appropriate cell responses. Most of immunoreceptors are regulated via tyrosine phosphorylations and their tyrosine motifs can be classified into three families: Immunoreceptor Tyrosine-based Activation Motif (ITAM), Immunoreceptor Tyrosine-based Inhibitory Motif (ITIM) and YxxM motif. Despite of its functional importance, the regulatory mechanism of these tyrosine motif phosphorylations remains unclear. Our previous work on T cell receptor (TCR) indicates that the phosphorylation of CD3ε ITAM is regulated by its dynamic interaction with the negative-charged phospholipids in the inner-leaflet of the T cell plasma membrane. Two tyrosine residues in the ITAM dip deeply in the hydrophobic core of the membrane bilayer before the receptor triggering. The sequestration of tyrosine residues into the plasma membrane thus represents a novel regulatory mechanism of receptor activation. Upon receptor ligation, the micro-environment of TCR changes rapidly, which results in the dissociation of ITAM from the membrane and the subsequent tyrosine phosphorylation by Lck. This tyrosine sequestration-release mechanism is not only limited to ITAM containing activating receptors, but also applicable to ITIM containing inhibitory receptors. Our data unveil the presence of an unexpected phospholipid regulation to membrane receptor activation.

Characterization of the two-protein complex that establishes lipid asymmetry at the outer membrane of Gram-negative bacteria

Chng Shu Sin

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Lipopolysaccharide (LPS) is a glycolipid found exclusively in the outer leaflet of the asymmetric outer membrane of Gram-negative bacteria. Proper placement of LPS at this location is critical for the outer membrane to function as an effective permeability barrier against toxic compounds, as well as for cell viability; thus inhibitors of this pathway could represent useful antibiotics. In *Escherichia coli*, two essential proteins LptD and LptE are responsible for establishing the lipid asymmetry of the outer membrane. In this seminar, I will talk about our work in characterizing the function and assembly of the two-protein LptD/E complex, and discuss possible mechanisms by which LPS is inserted into the outer leaflet of the outer membrane, including how this process may be regulated.

Qualitative and quantitative lipidomic assessment of human meibomian gland secretions

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Human meibomian gland secretions (MGS), or *meibum*, is a complex mixture of various lipids. They include cholesteryl esters (CE), wax esters (WE), (O-acyl)-omega-hydroxy fatty acids (OAHFA) and their esters as major components, and acyl glycerols (AG), free fatty acids (FFA), cholesterol (Chl), and other polar and nonpolar lipids – as minor ones. The chemical nature, (and the very presence) of certain lipid classes in MGS have been a matter of numerous research projects. The purpose of our study was to compare different experimental techniques suitable for lipidomic analysis of meibum, estimate their limits of usability, and conduct a comprehensive lipidomic analysis of human normal MGS.

Tested approaches included GC-MS, HPLC-MS, and BH-NMR. These procedures were used for structural evaluation and quantitation of unmanipulated samples of meibum collected from individual donors. The results obtained in our laboratory were compared with data obtained by other laboratories.

Over 120 individual molecular species of WE, ffl40 species of CE, and more than 30 species of OAHFA have been observed, structurally analyzed, and quantified in our studies. Quantitation of these lipids necessitated an elaborate approach based on the use of several authentic standards for each class of lipids. It has been determined that the average sample of normal (non-dry eye) MGS consisted of 42 ± 3 % (w/w) of WE, 33 ± 2 % of CE, with OAHFA present in the amounts of ffi10%. The other quantified lipid classes were: Chl (ffi1%), FFA (ffi0.5%), phospholipids (ffi0.1%), and squalene (ffi0.01%). Another minor group of lipids were AG (ffi5 species, with the overall content of ffi5%). At least 6 different Chl-esters of OAHFA were detected (~5% of MGS). A range of compounds reported in earlier publications were either not observed in our samples, or were shown to be contaminations that polluted the samples during their collection, handling, storage, and/or analysis.

A final lipid map shows the structures and molecular ratios of more than 200 individual meibomian lipid species that constitute >90% of total normal (non-dry eye) human meibum. This lays a foundation for further in-depth studies of biomolecular mechanisms of various ocular surface diseases, including ocular inflammation and the dry eye syndrome.

Algal lipids: topical and important

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Algae represent an extremely numerous and diverse group of photosynthetic organisms. Not surprisingly, their lipid compositions also reflect this diversity. In fact, even within classified groups (brown, red etc.) their lipid biochemistry can vary considerably. But this complexity enables algae to grow in all the major habitats on Earth and also offers potential advantages for exploitation.

In this talk I will describe two significant areas where algal lipid biochemistry is important. First, I will deal with the effects of climate change on the lipid metabolism of the green alga, *Scenedesmus obliquus*. Because such algae exist at the beginning of food webs, their changed lipid metabolism and composition has important implications for organisms further down the food chain. Second, I will discuss the potential for algae to be used as sources of biofuels. The predicted advantages and current problems will be highlighted as well as some examples of the 'state-of-the-art'.

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The alphabet of galactolipids in *Arabidopsis thaliana*

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Galactolipids constitute the major lipid class in plants. In recent years oxygenated derivatives of galactolipids have been detected. They are discussed as signal molecules during leaf damage, since they accumulate in wounded leaves in high levels. Using different analytical methods such as nuclear magnetic resonance, infra-red spectroscopy and high performance liquid chromatography/mass spectrometry (HPLC/MS) earlier reports focused on the analysis of either oxidized or non-oxidized species and needed high levels of analytes. Therefore a method for the analysis of the galactolipid subfraction of the Arabidopsis leaf lipidome by an improved HPLC/MSC-based method that is fast, robust and comparatively simple in its performance was established. Due to a combination of phase partitioning, solid phase fractionation, liquid chromatography and MSC experiments this method has high detection sensitivity and requires only low amounts of plant material. With this method 167 galactolipid species were detected in leaves of *A. thaliana*. Out of these 79 being newly described species. From all species the head group and acyl side chains were identified via MSC experiments. Moreover, the structural identification was supported by HPLC/time-of-flight (TOF)-MS and gas chromatography (GC)/MS analysis. The quantification of different galactolipid species that accumulated 30 min after a mechanical wounding in *A. thaliana* leaves showed that the oxidized acyl side chains in galactolipids are divided into 65% cyclopentenones, 27% methyl-branched ketols, 3.8% hydroperoxides/straight-chain ketols, 2.0% hydroxides and 2.6% phytoprostanes. In comparison to the free cyclopentenon derivatives, the esterifed forms occur in a 149-fold excess supporting the hypothesis that galactolipids might function as storage compounds for cyclopentenones. Additional analysis of the ratio of non-oxidized to oxidized galactolipid species in leaves of wounded plants was performed resulting in a ratio of 2.0 in case of monogalactosyl diacylglycerol (MGD), 8.1 in digalactosyl diacylglycerol (DGD) and 0.6 in the acylated MGD. This indicates that galactolipid oxidation is a major and rapid metabolic process that occurs class specific.

The MarVis software suite can be used to identify biosynthetic routes of metabolites. To identify the biosynthetic pathway of oxygenated galactolipids, galactolipid profiles of different lipid biosynthesis mutants were compared and the 1D-SOM arrays indicate that Arabidopsides may be synthesized via an enzymatic pathway which acts on lipid-bound fatty acids.

Acyl-CoA-binding proteins from *Arabidopsis* and rice

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Acyl-CoA-binding proteins (ACBPs) contain a conserved acyl-CoA-binding domain which binds acyl-CoA esters and can facilitate intracellular lipid transport. In Arabidopsis, a family of six genes encodes ACBPs which range from 10.4 kDa to 73.1 kDa, and have been subcellularly localized to different compartments in the plant cell. In rice, six genes also encode ACBPs (10.2 to 71.4 kDa). However the distribution of genes encoding ACBPs across 4 various classes differ between Arabidopsis and rice. The four classes consist of small (10 kDa) ACBPs, ACBPs with ankyrin repeats, ACBPs with kelch motifs and large ACBPs. *In vitro* binding assays have shown that recombinant ACBPs exhibit differential binding affinities to acyl-CoA esters implying that these ACBPs have non-redundant biological functions *in vivo*. Investigations using knockout/downregulated and overexpression lines of Arabidopsis ACBPs have shown that these ACBPs not only influence plant development but also stress responses.

Lipid metabolism and its gene regulation in model and non-model algae

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Algae and plants use solar energy and carbon dioxide to produce most of the biomass on earth, biomass can be used as a source of renewable energy and chemicals. Algae have an advantage over plants in this regard, as they can grow in a variety of aquatic and marine environments that are unsuitable for land plants. They do not suffer, therefore, from the problem of competition with food crops. There is, as well, enormous diversity among the algae that has barely been explored, making it quite likely that species and strains might yet be engineered or bred to have desirable industrial or aquacultural properties. However, much needs to be learned, still, about the metabolism and molecular genetics of these organisms. I will describe our efforts to understand gene expression and lipid changes that accompany the production of triacylglycerides in the model organism *Chlamydomonas reinhardtii*. I will also discuss what we have learned from the 1000 Plant Transcriptome project, which includes de novo transcriptome analyses of more than 100 diverse algae species. Lipid profiles for about 25 of these species have also been determined in collaboration with the Wenk lab (NUS). One of these species has been selected for further characterization because we have discovered that it has the unusual property of producing high levels of triacylglycerides under conditions of high salt, in addition to the more typical induction signal of low nitrogen. We expect that the existence of two distinct inducers will help us de-convolute the TAG production signaling cascade from the other physiological effects of nitrogen starvation and high salt stress.

Genetic strategies for oil yield improvement in oil crops and lipid productivity in algae

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Oil palm is among the largest source of edible oil which contributes to more than 20% of the world's production of oils and fats. Algae, on the other hand, have been considered one of the most promising feedstocks for biofuels. In both organisms, recent advances – particularly in the application of large scale quantitative trait loci (QTL) analysis and genetic association studies, in combination with the application of network biology and functional genomic tools – present opportunities to greatly improve productivity. In this presentation, the basic tools used by plant breeders and geneticists to identify and thereafter utilize markers linked or associated with key productivity related phenotypes (*e. g.* fruit size, oil yield, reduced height, disease and stress tolerance, etc in the case of crop plants) in the breeding programmes are discussed. Network biology tools (transcriptome, proteome, and metabolome analysis) used in tandem with the traditional genetic toolsets (linkage and association studies) can yield insights into underlying key processes controlling the complex multi-genic and interactive (gene–gene, gene–environment) nature of oil productivity.

Molecular mechanism of metabolic branching in the synthesis of glycosphingolipid

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Glucosylceramide (GlcCer) is the common precursor of hundreds species of Glycosphingolipids (GSLs). The synthesis of a given final GSL species primarily depends on the cell-specific expression of the dedicated enzymes, however the concomitant expression of enzymes devoted to the synthesis of different species of final GSL is commonly observed in a number of cell types thus leaving open the question as to whether the final GSL profile, in these cases, is stochastically determined or whether more active mechanisms exist. We have obtained a body of evidence indicating that the channelling of the common precursor GlcCer into the different final GSL species relays indeed on an active process that involves the mode of transport of GlcCer through the Golgi complex. In fact, from its site of synthesis at the *cis*-Golgi, GlcCer can be either transported via vesicular trafficking through the Golgi stack (undergoing processive glycosylation by the enzymes residing in the different Golgi cisternae) or it can be "shunted" to the trans-Golgi network (TGN) by the glycolipid transfer protein FAPP2, which operates the non-vesicular transfer of GlcCer (D'Angelo *et al*. Nature, 2007). We show that while ganglioside synthesis depends on vesicular trafficking of GlcCer through the Golgi stacks, globoside synthesis relies on the direct delivery of GlcCer to the TGN by FAPP2. We show that, both in diverse cell systems and in mice, the abrogation of FAPP2 function results in a specific globosides synthesis impairment with little if any effect on gangliosides production. Our results settle a precedent for different modalities to cross the Golgi complex serving differential cargo processing by Golgi enzymes.

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25-hydroxycholesterol is directly coupled to the interferon response and has antiviral activities.

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Oxysterols are potent oxidized derivatives of cholesterol which elicit important changes in many biological processes including cholesterol homeostasis, apoptosis, immune and inflammatory response. Previously, we have shown that sterol biosynthesis is regulated by the interferon response in the context of viral infection. Whether oxysterol regulation has a direct link with the interferon response is not clear. Here we provide evidence that, in response to interferon treatment, stat1 binds directly to the promoter of ch25h, the enzyme responsible for the production of 25-hydroxycholesterol (25-HC) in Bone Marrow derived Macrophages (BMDM). Subsequently, we demonstrate that 25-HC is secreted at very high levels in response to infection in BMDM. Notably, exogenous addition of a 25-HC blocks MCMV viral replication and egress ($IC_{50} \approx 1 \mu M$). Addition of 25-HC specific antagonist to conditioned media from MCMV infected macrophages increases viral replication indicating that physiologically secreted levels of 25-HC are sufficient to alter viral replication. In conclusion, this study indicates that oxysterol formation is directly linked to the interferon response and has antiviral activity at physiological levels.

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Lipidomics of host-pathogen interactions: human macrophage as a cellular system to study functional implications of lipid metabolism during infection

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The cell biology of intracellular pathogens (viruses, bacteria, eukaryotic parasites) has provided us with molecular information of host–pathogen interactions. As a result it is becoming increasingly evident that lipids play important roles at various stages of this intricate interaction between the pathogens and their hosts. Being positioned at the cell surface, lipids contribute to the interplay between host and pathogen, acting in first line recognition and host cell signaling during pathogen docking, invasion and intracellular trafficking. Serving as a basic building block of membranes and as an energy source, lipids are critical for the growth and replication of a pathogen.

Despite the growing appreciation of the relevance of lipids in infectious diseases, many gaps remain to be filled. Combining novel lipidomics approaches with synthetic chemistry, infection biology and molecular epidemiology, we aim to link changes in macrophage lipid metabolism, membrane trafficking and immunomodulation to defined pathogens including *Mycobacterium spp.*, as well as microbial lipids.

LCMS lipid profiling of intra-erythrocyte stages and intracellular organelles of *Plasmodium falciparum*

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Plasmodium falciparum is the major cause of malaria, a disease that afflicts more than 500 million people world-wide. The intraerythrocytic stages of *P. falciparum* exhibit a highly orchestrated developmental cycle within red blood cells (RBC) that takes approximately 48 hr. During this time, intracellular stages synthesize massive amounts of new membrane. There is accumulating evidence that intraerythrocytic stages are dependent on both the salvage of lipids from the host, as well as *de novo* synthesis of phospholipids and other lipid classes for intraerythrocytic proliferation. In this study we have used a LC-MS lipidomic approach to identify changes in the lipid composition of *P. falciparum*-infected RBC during this cycle, and to probe the lipid composition of the unique *P. falciparum* apicoplast organelle that is thought to harbor a number of prokaryotic lipid biosynthetic pathways. Lipids were extracted from synchronized *P. falciparum*-infected RBC and targeted lipid analyses undertaken using LC-MS/MS). Changes in phospholipid composition were detected in precursor scan and neutral loss scan modes and quantified by multiple reaction monitoring (MRM). These analyses highlighted significant changes in both the overall abundance and fatty acid composition of major phospholipid classes (PC, SM, PG, PE, PS, PI), ceramides and cholesterol esters in infected and uninfected RBC. Most striking was the accumulation of PI following the differentiation of ring stage to trophozoite stages. The *P. falciparum* apicoplast was purified using a new immunoaffinity protocol and subjected to comparable analyses. Lipid profiling revealed the differences in PI levels between the apicoplast and unfractionated parasite membranes. Unlike the plastids of photosynthetic apicomplexans the *P. falciparum* apicoplast lacked detectable galactolipids. Overall, these analyses suggest that intraerythrocytic stages are likely to be dependent on a number of pathways involved in lipid salvage from the host and remodeling/resynthesis of complex lipids.

Poster abstracts

Enhancing lipidomics by coupling HILIC with ion mobility MSE

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One of the main challenges for a global lipid analysis – lipidomics – is the separation of the wide array of lipid species present in biological samples. The ability to perform multi-dimensional separations in one injection prior to MS analysis could improve our ability to measure complex lipid mixtures.

Here, we first applied hydrophilic interaction chromatography (HILIC; BEH HILIC 1.7 μm, 2.1×100 mm) with a reversed phase solvent system (organic/aqueous) characterized by high organic mobile phase (>80% acetonitrile). This UPLC method was highly compatible with ESI and allowed to separate lipids by classes, according to their polar properties.

In addition to HILIC chromatography, we used an ion mobility TOF mass spectrometer to further discriminate lipids classes in their constituents based on the different cross collision sections. Lipid ions with different degree of unsaturation and acyl length migrate with characteristic mobility times due to their unique interactions with the nitrogen gas in the ion mobility cell.

To gain more structural information, lipids were analyzed using a parallel low and elevated collision energy analysis method to acquire both precursor and product ion information in a single analytical run. Because of the complexity of the biological lipidomes, the addition of mobility times as an orthogonal measurement to mass, retention times and MS/MS fragmentation provides complementary information regarding the analyte, which adds further specificity to the lipid identification and data interpretation.

Using this novel technological approach, we analyzed lipids extracted from various animal tissues, which were first separated by HILIC chromatography according to their classes (*e. g.*, ceramides (Cer), phosphatidylcholines and sphingomyelins) and, subsequentially, further separated in various components by ion mobility (*e. g.*, Cer d18:1 / 16:0, Cer d18:1 / 18:0, Cer d18:1 / 24:1).

In conclusion, the combination of HILIC with ion mobility separation delivers a multi-dimensional separation of complex biological mixtures, enhancing the lipidomic profiling.

Transmembrane mutation in FcγRIIb reveals the role of ceramide in phagocytosis and autoimmunity

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Phagocytosis is a phylogenetically ancient biological process employed for the protection of organisms from microbial infection and in the maintenance of tissue homeostasis through the removal of apoptotic and necrotic cellular material**.** The best characterized cellular receptors that mediate this process are the receptors for IgG termed FcγRs. FcγRs can be broadly separated into activatory or inhibitory receptors based on the presence of Immuno-Tyrosine Activatory Motifs (ITAMs) or Immuno-Tyrosine Inhibitory Motifs (ITIMs) in their cytoplasmic domains. The inhibitory receptor is proposed to regulate and dampen pro-inflammatory signaling and phagocytic activity mediated by the activatory receptors**.** This has important implications in autoimmunity as a single amino acid mutation in the inhibitory receptor, FcγRIIb, termed FcγRIIbCDCT has been identified that leads to hyperactive macrophages and is associated with the pathogenesis of autoimmune systemic lupus erythematosus (SLE). Moreover, the function of the mutated FcγRIIb is impaired because it fails to localize to sphingolipid rafts that from at sites of particle engagement and thus *cannot* regulate the activity of the activatory receptors that have coalesced here.

Mass spectrometry was employed to characterize the lipidome of phagosomal membranes extracted from U937 cells – a myelomonocytic human cell line that is differentiated into macrophages *in vitro*. This study demonstrates that phagosomes from U937 cells in which we have stably expressed the mutated inhibitory receptor, FcγRIIbCDCT exhibited half the concentration of ceramide than cells expressing its functional counterpart. It has been postulated that ceramide self-associates into small rafts, which traps cross-linked FcγRs. This study potentially enhances our understanding of the role of lipids in FcR-mediated phagocytosis and we propose that receptor cross – linking induced by binding of FcγRs to IgG coated latex beads may be the driving force for raft formation at sites of phagocytosis.

Characterization of a novel snake venom toxin from *Aipysurus eydouxi*

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Snake venom is a rich concoction of enzymatic and non-enzymatic proteins. cDNA library of venom gland not only gives us immense information about the composition of venom but also reveals novel toxins which are low in abundance. One such novel protein p156 was deduced from the cDNA library of the sea snake *Aipysurus eydouxi*. The amino acid sequence of this protein did not show significant similarity when blasted against the protein database. So, we expressed the protein p156 in *E. coli* bacterial cells and refolded it using redox buffer which was followed by its purification by Reverse-phase chromatography. After confirming the homogeneity of the protein by ESI-MS, we injected various doses of protein intraperitoneally in mice which caused writhing reactions and it was suppressed significantly when indomethacin was injected *i. p.* 30 min ahead of the protein injection. Also intraplantar injection of p156 protein in rat caused edema. This suggested that the protein is involved in inflammation and pain. So, we quantitated the prostaglandins present in the peritoneal exudate of mice at different time points and at various doses of p156 protein. The protein has a cytotoxic effect on RAW264.7 macrophages at higher doses. When 10 μM protein was administered to the cells then COX-2 enzyme was induced after 3 hr but there was no significant effect on activity or expression of COX-1 enzyme throughout. Nitric oxide production by macrophages due to p156 treatment was dose-dependent. Therefore, p156 protein is a pro-inflammatory protein from sea snake *Aipysurus eydouxi*.

High-throughput mass spectrometric assay for determining factors affecting substrate specificities of calcium independent phospholipases

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Cell survival depends on membrane phospholipid (PL) homeostasis and its ability in controlling lipid compositions which are tightly regulated by a plethora of calcium independent phospholipases (iPLAs) in mammalian cells. They are implicated in number of processes such as membrane PL maintenance, lipid remodeling and signaling. To get a better understanding of the principles underlying substrate specificities of putative homeostatic iPLAs, iPLA2 beta and NEST a truncated version of iPLA2 delta, a high-throughput mass spectrometric assay based on the use of exogenous isotope-labeled PLs was employed. It's a method that allows us to study the effect of acyl chain length, unsaturation of PLs and head group variability on their rate of hydrolysis in vesicle bilayers. Precisely it enables us to study the factors that influence the specificity of PLA2s by determining the kinetics of hydrolysis of a large variety of PL molecular species. We have successfully used this method in our earlier studies on secretory phospholipases (sPLA2) resulting in generating highly detailed information on its substrate specificity and the effect macrosubstrate properties had on it. In our present study we over-expressed iPLA2 beta and NEST as recombinant tagged proteins and sequentially purified them by affinity and ion-exchange chromatography that were then incubated with lipid bilayers composed of several different PL molecules to determine their relative hydrolysis by MS assay. Surprisingly our preliminary data regarding the substrate specificity profiles of iPLA2s were quite similar to that of the sPLA2s with the acyl chain length having a strong effect on the rate of hydrolysis and efflux being rate limiting. We also found that the rate of hydrolysis correlated with the hydrophobicity of the substrate. In conclusion our results suggest that efflux propensity is a key factor in the hydrolysis of membrane-bound PL molecules by iPLA2 family of proteins. In parallel we are carrying out atomistic simulations (CDOCKER) and docking studies (GOLD) to predict the properties and effects that lipid binding sites in the bilayer have on PLAs.

Towards determination of natural variation in blood lipids

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Recent advances in mass spectrometry complement existing platforms in providing rapid and sensitive methods for metabolite profiling. These emerging technologies allow better and more comprehensive detection of various metabolites including lipids. Many unanswered questions remain on the impact of genome natural variation in healthy population. The basis of biological variation cannot be completely explained by genomics, transcriptomics and proteomics. Lipidomics offers an avenue to complement these traditional technologies in helping to study lipid profiles across various conditions. Lipids have been found to be important in human health where dysregulation has been reported in pathological conditions such as neurological disorders, autoimmune diseases and cancer, among many. It is, however, imperative to understand how lipids vary in normal physiological settings. As such, this study aims to explore the range of biological variation in human plasma of various lipids including glycerophospholipids, sphingolipids and sterol derivatives in 360 healthy fasting Singaporeans. Targeted mass spectrometry using multiple reaction monitoring (MRM) was used to quantify over 200 individual lipid species. In a first step, variation that is introduced by sample pre-processing and instrumentation, *i. e.* technical variation, has been carefully considered. Ultimately, this work aims to quantify biological variation and link metabolite patterns with genomic and transcriptomic data to provide a biological meaning on underlying mechanisms.

Tracking sphingosine metabolism and transport in sphingolipidoses: no evidence for NPC1 as a sphingosine transporter

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The late endosomal/lysosomal compartment (LE/LY) plays a key role in the breakdown of sphingolipids. The sphingolipid acid hydrolases are rather well characterized, while less is known regarding how sphingolipid degradation products exit the LE/LY. The last step in the LE/LY sphingolipid degradation is catalyzed by acid ceramidase, releasing a fatty acid and sphingosine. At the acidic pH of the lysosome, the primary amino-group of sphingosine is protonated, reducing its ability to pass through the LE/LY membrane. The mechanism(s) for sphingosine egress form LE/LY is unknown but Niemann-Pick C1 protein (NPC1) has been suggested to be involved. Here, we have devised a method for tracing the metabolic fate of lysosomally targeted sphingolipids. The method was evaluated in cells with known defects in sphingolipid catabolism, and then used to test the hypothesis that NPC1 is involved in egress of lysosomally generated sphingosine. 3-[DH]-sphingomyelin, 3-[DH]-ceramide and 3-[DH]-sphingosine were generated and targeted to cells in low-density lipoprotein particles in order to biochemically track

sphingolipid metabolism in the LE/LY. In addition, we synthesized a fluorescently labelled sphingosine derivative to visualize sphingosine trafficking and distribution in cells, as well as to correlate these findings with the biochemical observations. The probes traced the LE/LY sphingolipid degradation pathway as suggested by 1) the depletion of 3-[DH]-ceramide and 3-[DH]-sphingosine generated from 3-[DH]-sphingomyelin upon acid sphingomyelinase knockdown, and 2) the accumulation of 3-[DH]-sphingomyelin-derived 3-[DH]-ceramide and depletion of 3-[DH]-sphingosine upon acid ceramidase knockdown. NPC1 silencing did not result in the accumulation of 3-[DH]-sphingosine derived from 3-[DH]-sphingomyelin or 3-[DH]-ceramide, nor in defective resynthesis of 3-[DH]-ceramide from 3-[DH]-sphingosine. The fluorescent sphingosine-BODIPY displayed a similar distribution wild type and NPC1 knockdown cells. This study suggests that the endomembranes of NPC1-deficient cells have an increased capacity for accommodating sphingosine, without restricting its efflux from the LE/LY. The method presented here can be useful for screening for lysosomal sphingosine transporter proteins.

Lipidomic analysis of multi-drug resistant bacteria

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The alarming increase in resistance of bacteria to commonly used antibiotics contributes significantly to patient morbidity and mortality, which highlights the importance of continuing to develop novel antibacterial agents to treat these life threatening multidrug resistant infections.

The Cooper Group at The University of Queensland has been developing several series of new antibacterial drugs with a particular focus on a novel approach that targets the bacterial membrane over mammalian membranes. In proof of concept studies, new glycopeptide derivatives have been synthesized that have potent activity against drug resistant strains of the Gram-positive bacteria *Staphylococcus aureus* (*e. g.* MRSA), *Streptococcus pneumoniae* and *Enterococcus faecalis* (*e. g.* VRE). During the course of these studies it became obvious that there had been no detailed analysis of bacterial-derived phospholipids amongst the various resistant phenotypes.

We therefore undertook a comprehensive study of the phospholipids of the various phenotypes of *S. aureus*, *S. pneumoniae*, *Streptococcus pyogenes*, and *E faecalis/faecium*, which are pathogens responsible for most Gram-positive skin and skin structure and pneumonia infections, and *Clostridium difficile*, which is associated with severe diarrhoea. The lipid profiles of these bacteria were obtained using standard ESI shotgun methods and HILIC-based LC-MS analyses. The data obtained from these studies will not only provide a valuable insight into the lipid relationships between pathogens and their resistant strains, but offer opportunities for drug design more suited to combat the emergence of drug resistant bacteria.

Lipid remodeling upon epithelium formation

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Upon differentiation cells undergo drastic morphological and metabolic changes. Most likely these metabolic changes are associated with lipidomic variations. We tested this hypothesis on cells undergoing epithelial differentiation. In this system, MDCK cells are seeded into gelified extracellular matrix to form a cyst after a few days. A cyst is a spherical epithelial structure with the basal side facing the extracellular matrix and the luminal side being internal to the sphere. We conducted lipid mass spectrometry on cysts at different time points after cell seeding. We indeed observed variations in their glycerophospholipid and sphingolipid composition over time. Not only levels of certain lipid species vary but the acyl chains of phosphatidylcholine and phosphatidylinositol elongate and become more unsaturated. In order to understand the significance of these lipidomic variations we used two approaches. On one hand, we used inhibitors to perturb the glycerophospholipid biosynthetic pathway upon epithelium formation. By doing so we observed that inhibition of the phosphatidic acid phosphorylase (PAP) activity after addition of propranolol disturbed the cyst lumen formation. On the other hand, we affected the cellular lipid composition by feeding the cells with various fatty acids. We confirmed by mass spectrometry that the chain length and saturation of glycerophospholipids directly depend on the source of fatty acids found in the milieu. Cells fed with linoleic or linolenic acids underwent normal differentiation into epithelium, whereas stearic acid feeding generated epithelium with defective adherent junctions. Our data suggest that phosphatidic acid, diacylglycerol and/or downstream products as well as the unsaturation of lipids actively contribute to the epithelium formation.

Lipidomics analysis of the Tyrolean Iceman

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The Tyrolean Iceman, also known as Ötzi, was found in the Tyrolean Alps in 1991. Europe's oldest natural mummy, he died around 5300 years ago and his body was remarkably well-preserved probably because it was covered in ice shortly after his death. Such discoveries are rare and can give us much information regarding lifestyles, skills and even diet of ancient humans. Previous analyses of the Iceman's tissues have provided information regarding his health condition, the way he died and even what his last meal was.

Recently, his stomach, long thought to have decomposed, was discovered to be still present, and new samples became available that could help shed more light on Ötzi's lifestyle.

In order to extract new information from these samples, we will use an array of chromatographic and mass spectrometric techniques including thin layer chromatography, normal and reverse phase ChipLC-QToF-MS/MS and GC-MS/MS.

Biochemical lipidomics of erythrocytes from patients with Schizophrenia

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Blood is a fluid organ whose major constituents are erythrocytes, leukocytes and platelets. Different human blood cells have unique lipid compositions which match their functional requirements. Composition and organization of these lipid inventories are tightly regulated. Therefore captured abnormalities can act as useful bio-correlates for physiology and disease. Moreover, blood collection being a semi-invasive routine procedure, makes it favourable in a clinical setting. Lipids are dynamic regulators with diverse physiological functions which consist of controlling of transcription and translation, signal transduction, cell-cell interactions and acting as biological messenger. In the past, the study of lipids was hampered by analytical limitations. Today, with advancement and development in mass spectrometry, comprehensive analysis of various lipid classes can be readily accomplished through a plethora of analytical methods.

Schizophrenia is a serious mental illness with a lifetime prevalence of approximately 1%. Currently, there is no validated objective laboratory measure that is used routinely in clinical care. The diagnosis and monitoring of disease is almost exclusively based on history and clinical observations. The present study reveals an increase in morphologically disorganized forms of erythrocytes in the blood of schizophrenia patients. Furthermore, lipidomic profiling of erythrocyte membranes showed altered lipid levels. In total, 25 lipid species were identified that could act as potential multi-parameter markers for the detection of schizophrenia irrespective of antipsychotic treatment. Using linear discriminant analysis, the predictive model showed an accuracy of close to 87%, and a leave-one-out cross-validation model had an accuracy of 76%. Choline lipids formed major component of these markers, indicating the involvement of choline metabolic pathways in schizophrenia. Thus, morphological variations of erythrocytes in conjunction with altered lipid profiles could form a basis for the diagnosis of this disorder.

Screening and characterization of *Rhodococcus opacus* Tn5 mutants for biofuel production

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Rhodococcus opacus, a prolific producer of triacylglycerides, potentially can be exploited for the production of biofuels from several renewable resources and waste streams. Although previous attempts have tried to understand the metabolism of storage triacylglycerols in *R. opacus*, a clear understanding of the genetic elements has yet to be properly elucidated. A key reagent in studying gene function is a transposon library allowing for the identification of the underlying molecular mechanisms of lipid biosynthesis. Tn5 transposon mutants have been developed and are currently being screened for increased triacylglycerides (TAG) accumulation as compared to wild-type *R. opacus*. Through sequencing of TAG hyper-accumulating mutants we aim to identify genes related to TAG biosynthesis. Specific lipid profiles are further explored through mass spectrometry in identifying target biomass end products. An optimized biocatalyst can be engineered from this genetic and lipidomic information with a specific multi-parameter footprint related to activity, stability, specificity, and efficiency. A model developed from this microbial system to biofuel will be constructed to map a potentially efficient conversion pathway to renewable energy.

Keywords: *rhodococcus*, biofuels, lipidomics

An analysis platform for lipidomics

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In order to store and analyze consistently large quantities of MS data for different projects within LipidX, we conceived and implemented a single platform integrating the most common steps of the lipidomics research strategies. Notably, this platform supports both the exploratory phase, to find new lipid species of biological interest (OrbiQuant application) and the quantification phase to obtain precise profiles for lists of known lipids (MRMQuant application). It also provides a common way to store, manage and analyze experimental data acquired from different machines located in different laboratories for all LipidX members, and guarantees data integrity and consistency across experiments and over time.

Currently, 24 users access, analyze or upload data on the platform under 9 different roles. A total of 1782 lipids were added in the LipidX database and 549 manual annotations on lipid compounds have been done and stored in the database. 364 experiments have been uploaded from 4 machines located in different places (2 TSQ machines and 1 VARIAN 320 and 1 LTQ Orbitrap) and on cell extracts of 10 different organism types. Additional 310 experiments were uploaded on a prototype of the analysis platform. All these experiments represent a number of about 75 000 samples (technical and biological replicates comprised). Since now, 306 analysis of Multiple Monitoring Reaction (MRM) experiments have been computed, comprising 164 using the MRMQuant application of the platform. 62 OrbiQuant runs have been run so far to identify relevant changes between full scan profiles of different lipid extracts.

Application of ^{1}H and ^{13}C NMR Spectroscopy in the investigation of the time and temperature related conversion of punicic acid to its geometric isomers in pomegranate seed oil

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Pomegranate seed oil (PSO) is a rich source of polyunsaturated acids mainly conjugated linolenic acid isomers (CLA's). Among the four geometric isomers of octa-9,11,13-trienoic acid the cis, trans, cis isomer (punicic acid) is the most important fatty acid of the pomegranate seed oil responsible for its unique cholesterol lowering and anticancer effects (1–2).

In the present study we wish to report the application of BH and BDC NMR spectroscopy to evaluate the time and temperature related conversion of punicic acid to its geometric isomers in PSO.

Our results showed that punicic acid is the most abundant CLA in fresh pomegranate seed oil constituting 92% of total CLA's followed by cis, trans, trans isomer (α-eleostearic acid) with %5.5 and trans, trans, cis isomer (catalpic acid) with %2.5. By storing PSO in room temperature for one year a significant decrease in punicic acid and a major increase in α-eleostearic acid and catalpic acid levels were observed. Furthermore a new all trans isomer (β-eleostearic acid) was detected in PSO. Punicic acid, α-eleostearic acid, catalpic and β-eleostearic contents in one year's old PSO were %37, %23, %18 and %22 respectively. A quite similar pattern was observed after the heating of PSO at 120 °C for 0.5 hours. Increasing the heating time had little additional effects on trans isomers levels.

This study showed that punicic acid has a high tendency for conversion to its trans isomers, hence it is recommended that only fresh PSO should be used for food purposes and it's punicic acid content must be determined prior to use especially for pharmaceutical applications.

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Targeted and untargeted lipidomics analysis of human synovial fluid

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In this presentation we will focus on a new lipidomics LC-(MS/MS) platform incorporating a fast scanning ion trap for simultaneous data acquisition in the positive and negative electrospray mode. Next to the basic analytical characterisation of the platform, we demonstrate its applicability providing a first description of the lipid species and lipid mediators present in human synovial fluid of osteo (OA)- and rheumatoid (RA)- arthritis patients. We identified substantial differences between the patients, particularly with respect to poly-unsaturated-fatty-acids (PUFA) and their hydroxy products. As the aforementioned substances are the precursors and biomarkers for the downstream production of numerous potent lipid mediators such as leukotrienes, resolvins, lipoxins and others, these findings might lead to the definition of novel disease progression markers. This encouraged us to conduct a detailed investigation of the lipid mediators present in the synovial fluid of both OA and RA patient samples. In this second part of the lecture, we will present a targeted lipidomics platform based on a QTrap mass spectrometer for the highly sensitive determination of several biochemically important lipid components. We will emphasize the mass spectrometric identification and quantification of several of the aforementioned substances as well as their biological function. With the described platform we carried out a detailed investigation of the lipid mediator profiles, present in the synovial fluid of arthritis patients. The obtained results clearly revealed differences between the investigated patients. In addition to the mass spectrometric data we will also emphasize the biological importance of our findings and propose future advancements and necessities in the field.

Development of anti-human monoclonal antibody against the alcohol biomarker phosphatidylethanol

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Chronic alcohol consumption is associated with increased risk of gastrointestinal cancer. High concentrations of ethanol trigger mucosal hyper regeneration, disrupt cell adhesion, and increase sensitivity to carcinogens. Most of these effects are thought to be mediated by acetaldehyde, a genotoxic metabolite produced from ethanol by alcohol dehydrogenases. Several attempts have been made to develop reliable laboratory tests to detect long-term heavy alcohol drinking. These efforts have focused on a set of biomarkers that are both direct and indirect products of ethanol metabolism. Recently, phosphotidylethanol (PEth) has been proposed as a promising new marker for detecting long-term alcohol abuse. PEth is formed via a phospholypase D (PLD) catalyzed transphosphatidylation reaction in the presence of ethanol from phosphatidylcholine (PC). To detect PEth, we currently need to employ highly expensive and time consuming instrumentation, and the unavailability of a rapid assay to detect this lipid hampers its utility as a biomarker. To overcome this limitation in PEth analysis, we aimed to generate a monoclonal antibody against PEth. Using an in-house Phage-antibody library we successfully generated several antibodies against PEth. Our results show that PEth antibodies can be generated with fine specificity for Phosphatidylethanol and these do not cross react with similar molecules such as PC or phosphatidic acid (PA). This antibody can detect nanogram levels of PEth by ELISA, and useful in ELISA, dot blot, TLC immunodetection and FACS analysis. Finally we induced PEth in human (*in vitro*) and rat blood (*in vivo*) and found that our antibody is able to detect the PEth in inducible samples. Our primary hypothesis is that ethanol induced PEth in the blood is deposited on the leukocytes, which then is detected by our antibody. So, we conclude that using Phage display technique we have developed very specific antibody against PEth which can be used as a diagnostic tool for ethanol biomarker.

Imaging the "Turtle" – A metabolic labelling approach for targeting inositol lipids

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The molecule myo-inositol ("turtle") is extremely crucial to various cellular processes. Absence of inositol biosynthesis or unavailability from the media causes auxotrophy in eukaryotes and some prokaryotes. Phoshpatidylinositol and Phosphoinositide lipids form components of cellular membranes and along with free inositol phosphates, participate in various signalling mechanisms. Higher inositol sphingolipids and glycosyl-phosphatidylinositol (GPI) anchors are known to be involved in host-pathogen interactions and in interactions of the cell with the extracellular environment.

Inositol lipids have mainly been studied through expression of fluorescent lipid binding protein domains (PH, FYVE) which raise concerns over non-specificity and non-lipid interactions. We explore a method using the innate variations in enzyme-substrate specificities, particularly that of the primary inositol lipid synthesizing enzyme Phosphatidylinositol synthase (PIS1) for generating metabolic synthetic lipids. *Myo*-inositol was modified with an azide bio-orthogonal group which can then be targeted with an alkyne functionality through azide-alkyne Huisgen cycloaddition (Click reaction). *Saccharomyces cerevisiae* (budding yeast), was selected as the model organism to study the effects of the inositol analogues on growth, their interactions with the cellular environment and analyze their incorporation into inositol lipids. Inositol analogue incorporation was ascertained through mass spectrometry of the yeast lipid extracts and analyzed by click chemistry.

The above method gives insight into the substrate specificities of enzymes involved in inositol lipid biosynthesis. Modified lipids were tagged intracellularly and in lipid extracts with alkynated fluorophores. Azido-inositols thus show potential as a tool for studying the localization and trafficking of inositol lipids.

The ceramidome and toll-like receptor signaling

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Toll-like receptors (TLRs) are transmembrane proteins residing either at the plasma membrane of cells or within endosomal membranes to sense conserved pathogen associated molecular patterns (PAMPs). TLRs, mainly present on immune cells like macrophages and dendritic cells, not only trigger immediate alarm pathways but also prime antigen-specific adaptive immunity and are additionally associated with inflammatory diseases. Thus, TLR signaling needs to be precisely regulated to warrant anti-microbial defense without hyper inflammation or erroneous activation. Membrane partitioning has been shown to affect signaling by immune receptors in numerous cases and enzymes involved in the sphingolipid and ceramide biosynthesis play a general role in the modulation of membrane structures and signal transduction. Our own proteomic survey of TLR interactors has identified several GPI-anchored proteins and lipid-modifying enzymes (Baumann *et al.*, JEM 2010; unpublished observations). Could sphingolipid metabolism, sphingolipid signaling and membrane partitioning intersect with TLR-signaling and modulate inflammation? We set out to perform a multi-scale survey of the role of sphingolipids and ceramides on TLR signaling. A bio- and chemoinformatic-assisted examination of the knowledge domain identified enzymes and lipid-binding proteins expressed in murine macrophages, many available purified lipids and several chemical compounds that impinge on sphingolipid metabolism. Hence, we started a multiplexed shRNA-based loss of function campaign and chemical perturbation screen on robust TLR-specific quantifiable signaling readouts as well as transcriptome analyses. Based on these results we will prioritize proteins to further validate by interaction proteomic analysis and overexpression studies as well as loss-of-function cell lines that will be subject to global mass-spectrometry-based lipidomics. Resulting data will be used to generate computational models and precise hypotheses will be tested by dual perturbation and rescue experiments (drug-shRNA; drug-ceramide, shRNA-ceramide) to obtain the logical map of the effects of sphingolipid and ceramide pathways on TLR signaling. Subsequent work will investigate the role of individual components on the organism level.

Lipid perturbations in dysfunctional tear syndrome

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Secretion from the meibomian glands, or the meibum, constitutes the cardinal source of lipids for the tear film lipid layer (TFLL) in humans. Lipidomic analyses of the human meibum have revealed that a significantly lower level of triacylglycerides (TAG) ($p < 0.05$) was observed in patients from moderate category of dry eye compared to the mild category classified based on the ocular surface disease index (OSDI). Notably, a number of O-acyl-w-hydroxy fatty acids (OAHFA) displayed a consistently decreasing trend with increasing disease severity. Meibum-derived lipid markers indicative of dysfunctional tear syndrome (DTS) progression, however, are circumvented by the assumption that meibomian lipids are fully incorporated into the TFLL without proportional alterations of the various lipid classes. Nevertheless, the high proportion of neutral lipids in the human meibum and the predominantly aqueous nature of the human tear film imply that a selective enrichment of polar lipids might occur in tears. Furthermore, a comprehensive lipidomic profiling of human tears would greatly facilitate the identification of other minor sources of tear film lipids apart from the meibomian glands. Tear lipid biomarkers can also potentially offer a closer reflection of disease pathophysiology and display better correlation to clinical tests currently used for dry eye diagnosis, such as the Schirmer's test and the tear film breakup time, which are developed largely based on tear film biophysics. Nonetheless, the appreciably lower lipid concentration in human tears compared to the meibum, as well as the limited amount of basal tears that can be obtained from individual subjects, present a new level of challenge for the field of analytical lipidomics that warrants the development of state-of-the-art analytical approaches to achieve the ultimate goal of comprehensively profiling the lipids in tears.

Global metabolite profiling of the *Medicago truncatula* mutant under salt condition using gas chromatography-mass spectrometry

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Metabolite profiling of *M. truncatula* mutant was performed using gas chromatography-mass spectrometry (GC-MS) coupled with multivariate statistical analysis. Comparative evaluation was carried out to investigate the difference of metabolome according to various gene types, cultivation conditions, and plant organs. There are totally 8 groups of *M. truncatula*. Eight groups are composed of wild type (WT)–control–root, WT–control–shoot, WT–salt–root, WT–salt–shoot, mutant type (MT)–control–root, MT–control–shoot, MT–salt–root, and MT–salt–shoot. Δ-1-Pyrroline-5-carboxylate synthase (P5CS) gene was genetically manipulated in MT. P5CS is a key enzyme for the rate-limiting step of proline biosynthesis. The rate-limiting step of proline biosynthesis from glutamate is a two-step reaction that is ATP-dependent phosphorylation of glutamate to γ-glutamyl phosphate and then the NADPH-dependent reduction to glutamate semialdehyde (GSA). Salinity is one of the major environmental stress that changes the metabolites of plants by inducing formation of reactive oxygen species (ROS). Metabolic profiles of each sample obtained from GC-MS analysis were further analyzed using principal component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA). This study shows the content of lipids, amino acids, and other metabolites are significantly differed between wild type and P5CS3 gene mutant of *M. truncatula* cultivated in control and salt conditions.

Keywords: *Medicago truncatula* mutant, GC-MS, metabolite profiling, multivariate statistical analysis

Cell cycle lipidomics

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Lipids play essential roles in many cellular processes, which are functionally linked to the cell cycle. Although many efforts have been put in to understand how lipids are regulated in the cell cycle, no high-resolution lipid profiling of the cell cycle has been reported. Using HeLa cells as a model, this study aims to provide novel insights into variation of a cellular lipidome during the cell cycle. Cells were synchronised at G1/S using aphidicolin, a reversible inhibitor of nuclear DNA replication. The cells were then collected at G1/S and G2/M stages, and lipid extraction was carried out using modified Bligh and Dyer's method. The samples were subjected to lipid analysis by liquid chromatography-electrospray ionisation mass spectrometry (LC-ESI MS) using multiple reaction monitoring (MRM). In total, 237 lipids, including phosphatidylcholine (PC), phosphatidylglycerol (PG), phosphatidylinositol (PI), phosphatidylserine (PS), phosphatidylethanolamine (PE) and sphingomyelin (SM) were quantified against spiked internal standards for the respective lipid classes. Cholesterol and its derivatives were also analysed. Here, we would present and discuss the global changes in the cellular lipidome as the cells progress in the cell cycle.

Enzyme in bioactive lipid signaling – from structure to mechanism and drugs

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It is in our interest to study the important players involved in lipid signalling pathway using X-ray crystallography and other biophysical techniques. We aim to investigate the structure of these proteins and relate to the mechanics for its function, followed by application of fragment based drug design to propose novel lead compounds inhibiting or enhancing the protein role. One of our present targets is Lipocalin-Prostaglandin D synthase, a protein downstream of arachidonic acid pathway that converts Prostaglandin H2 (PGH2) to Prostaglandin D (PGD), a well known inflammatory mediator. It also acts to transport lipophilic compounds like biliverdin, all-trans-retinoic acid and bilirubin. We aim to understand how catalysis occurs as well as the dynamics that regulates the substrate/ligand entry and exit of the protein; hence exploring new grounds for specific and effective drug design. Wild type unbound human PGDS crystal structure was solved at a resolution of 2.0 Å (PDB id: 2WWP). The protein shows increased stability in thermal shift assay with its substrate analog (U-46619), a known lipophilic ligand, all-trans-retinoic acid and its product, Prostaglandin D. This results in successful attempt of co-crystallization with substrate analog giving structure of 1.7 Å resolution however the density at ligand binding site was not clear. We then complement the studies with NMR experiments to capture the dynamics of protein-substrate formation. A preliminary 2D HSQC experiment shows chemical shifts upon titration with its substrate analog. Upcoming work would be to focus on solving the complex structure of protein-substrate/ligand to elucidate the protein binding specificity and potential drug binding site.

A Sensitive and efficient method for S1P quantification in biological samples by chip-based LC-MS

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Sphingosine-1-Phosphate (S1P) is an important bioactive lipid mediator and a normal constituent of human plasma. S1P is synthesized from sphingosine by the enzyme Sphingosine Kinase and its biological activity is modulated through binding to specific receptors. S1P plays important physiological roles in the regulation of cell growth, differentiation, motility and survival; in addition it participates in pathological conditions like autoimmunity, cancer, angiogenesis and myocardial infarction. Thus S1P gained clinical importance as biomarker for diseases. For the detection of S1P in biological matrices we developed a simple, quick, sensitive and reliable assay by using an Agilent HPLC-Chip MS system after derivatization of the sample. Preliminary analysis showed that this new method is a valuable tool for the detection of S1P from small amounts of human and mouse plasma samples.

An identification-quantification lipidomics workflow utilising off-line enrichment and class specific separation of phospholipids in human plasma

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The chemical complexity of lipids present demanding analytical challenges; from the sample preparation stage right up to the analytical techniques used to identify and quantify key lipids. Today, many variations to the Bligh and Dyer method are used for total lipid extraction and purification, and just as many for mass spectrometric analysis. Recent advances in lipidomics has made use of developments in chemistries and instrumentation, most notably the use of off line enrichment or solid-phase sample preparation products and the coupling of ultra-performance liquid chromatography with mass spectrometry. Here, we present a complete phospholipid analysis workflow from extraction to separation, identification and quantification of the phospholipids.

For sample preparation, an off-the-shelf bioanalysis sample preparation plate was repurposed for the extraction of phospholipids. The optimised method extracts the phospholipids in two fractions; one containing the more highly abundant PCs and SMs and the other containing low abundance phospholipid classes. The fractions were combined at appropriate dilutions optimised for the experimental dynamic range of the MS detector. Samples were then analysed by HILIC UPLC-MS giving class distinct separation of the phospholipids in a 10 minute gradient run. A database of mass transitions, validated by accurate mass Q-ToF data, was used to program a tandem quadrupole MRM method. This method was then deployed for the quantification of phospholipids in human plasma samples. A matching data processing method was also created for the high throughput quantification of all the phospholipids in a sample. This data can then be exported for further statistical analysis.

The streamlined workflow described for phospholipids above could be applied to the analysis of various lipids in other biological matrices with minimal method modification. Application of this workflow could also greatly accelerate the discovery of key lipids important in the pathophysiology of diseases.

Fatty acids & hormones composition profiles during oil palm fruit mesocarp development

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Oil palm is one of the most productive oil producing crops and can store up to 90% oil in its fruit mesocarp. Accumulation of oil in mesocarp tissue occurs during fruit development. Oil palm fruit undergoes different developmental stage from 8 week after anthesis (WAA) for cell growth and expansion until 22 WAA into a fully grown mature mesocarp. Deposition of oil, fatty acids compositions, and hormones of the oil palm fruit mesocarp were investigated to gain a better insight into the accumulation of these components. Plant hormones are key regulators of many fruit processes, including oil accumulation. Hence, in this study, the lipid and hormones metabolites from oil palm mesocarp during mesocarp tissue development were profiled by using Gas Chromatography–Mass Spectrometry (GCMS) and Liquid Chromatography–Mass Spectrometry (LCMS) respectively. Total lipids and hormones were extracted from different stages of fruit development (namely 12, 14, 16, 18, 20, and 22 weeks after anthesis (WAA)). Results have shown that the lipid content in mesocarp increases from 16 WAA and this correlates also with the abundance of fatty acid composition detected by GCMS. Both palmitic and oleic acid is found to increase about 3-fold from 16 WAA to 18 WAA. The concentration of abscisic acid increases from the 16 WAA onwards until 20 WAA. Interestingly, the trend of abscisic acid is same as oil accumulation in mesocarp. The results of this study indicate that developing mesocarp revealed significant changes in the lipid and hormones profiles during fruit development.

Aggregation of FcγRI triggers formation of polyunsaturated phosphatidic acids in U937 cells

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Aggregation of Fragment crystallizable receptors for IgG, FcγRs, is linked to a range of cellular and immunological responses including receptor mediated endocytosis of immune complexes, degranulation, activation of respiratory burst and secretion of pro-inflammatory cytokines. FcγRI (CD64), the principle high affinity activatory receptor for human IgG, signals through an ITAM containing FcRγ-subunit and is coupled to PLD1. FcγRIIA, the principle low affinity, activatory receptor for IgG signals through its own non-canonical, intracytoplasmic ITAM motif and is coupled to PLCγ1. FcγRIIB is an inhibitory Fc-receptor that signals through an ITIM motif and modulates the activity of other FcRs. Phosphatidic Acid (PA) metabolism and signaling associated with FcγR biology has not been extensively characterized. In this study we employ liquid chromatography/electrospray ionization MS (LC/ESI-MS) to profile and compare PA lipid mediators generated through the activation of FcγRI or FcγRIIA in the presence or absence of FcγRIIB. We show that FcγRI aggregation in U937 cells induced the formation of phosphatidic acids indicating activation of PLD1. Interestingly, polyunsaturated phosphatidic acid species were significantly higher in U937WT cells versus U937 expressing FcγRIIB (CD32BICDC cells), suggesting a link between FcγRI and FcγRIIA signaling. We have investigated this link through inhibition of PLD1 (using VU0359595), PLCγ (using U73122) and DGKI (using D5919). Intracellular calcium was utilized to ascertain the specificity and optimization of experimental conditions for the inhibitors. We show that polyunsaturated PA levels are decreased in the presence of U73122 but unaffected by D5919. This suggests that polyunsaturated PA species are derived from PLCγ and DGKε catalyzed reactions and that FcγRI aggregation may also activate FcγRIIA coupled PLCγ signaling.

How do lipids organize and move in membranes? Parallel AFM and single-molecule diffusion analysis of fluorescent lipid analogues and sphingolipid-tracing peptide probes

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Suspended and supported bilayers are well-characterized, simplified model systems of biological membranes whose purpose is to compartmentalize and functionalize fundamental processes in living organisms. Our focus is to devise and characterize tools to elucidate the dynamics of "lipid rafts", *i. e.* nanoscaled, ordered domains, enriched in sphingolipids, (including gangliosides) and cholesterol, which are presumed to further compartmentalize and functionalize raft-localized membrane proteins. We study mica- or glass-Supported Lipid Bilayers (SLBs) and Giant Unilamellar Vesicle (GUV) systems to characterize the phase separation dynamics of various multi-component lipid mixtures and correlate these with specific diffusion behaviors of lipid- and peptide-based fluorescent probes.

Atomic Force Microscopy (AFM), operating in aqueous solutions, reveals phase separation characteristics of SLBs at well below the resolution limits of conventional fluorescence microscopy, making it possible to observe nanodomains. In parallel, we are establishing diffusion behavior and dynamics of lipid-conjugated dyes and fluorescently labelled raft probes by fluorescence Single Molecule Tracking (SMT) in TIRF (Total Internal Reflection Fluorescence) mode on SLBs.

We compare the diffusion and phase separation behavior of probes in supported bilayers to their behavior in suspended bilayers by creating GUVs of the same lipid composition. In addition to yielding important information about the partitioning behavior of the dyes, fascinating effects on phase separation dynamics and membrane morphology has been observed in GUV membranes perturbed by weak osmotic gradients (*e. g.* biologically reminiscent morphological transformations such as endo- and exocytosis, budding, membrane fission and fusion and raft formation). As we come to better understand our artificial systems and are able to model live cells more accurately, we also increase the predictive power of the combined SLB and GUV data to associate a given diffusion behavior with particular phase separation and organizational characteristics.

Strong preferences of dopamine and L-dopa towards lipid head group: Importance of phosphatidylserine and its implication for neurotransmitters metabolism

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The interactions of the neurotransmitter dopamine and its precursor L-dopa, with membrane lipids, were investigated using atomistic molecular dynamics simulations. The results obtained indicate that dopamine and L-dopa strongly interact with the lipid head groups *e. g.* via hydrogen bonds. These interactions anchor the molecules to the membrane interfacial region. The strength of this bonding is dependent on lipid composition – the presence of phosphatidylserine leads to increased bonding strength with a lifetime much longer than the timescale of our simulations. The high membrane association of dopamine and L-dopa both, extracellularly, favours the availability of these compounds for cell membrane uptake processes and, intracellularly, accentuates the importance of membrane-bound metabolizing enzymes over their soluble counterparts.

Atomistic simulations indicate cardiolipin to have an integral role in the structure and function of the cytochrome *bc1* complex

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The reaction mechanism of the cytochrome bc1 (cyt-*bc1*) complex relies on hydrogen and electron transfers from ubiquinol, which in turn generates a proton gradient across the mitochondrial membrane. Cardiolipins (CLs) have been suggested to ensure both the structural integrity of the complex and to take part in the proton uptake. We study the issue by simulating the entire cyt-*bc1* dimer of Rhodobacter capsulatus using all-atom molecular dynamics. In the 200 ns simulations CLs and phospholipids enter the dimer interface and position themselves close to the higher potential heme groups in the Q_i-site. The CL molecules were able to occupy a central location inside the cavity, which is consistent with previous X-ray crystallographic results. The changes in the electrostatic potential support the idea that the lipids influence the transfer processes. Overall, the conserved positioning of CL inside the Q_i-site suggests that it has a key role in the cyt-*bc1* complex function.

Determination of punicic acid content in pomegranate seed oil prepared from old pomegranate seeds by ^{1}H and ^{13}C NMR Spectroscopy

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Among several conjugated linolenic acid (CLA's) isomers cis, trans, cis octa-9,11,13-trienoic acid (punicic acid) is the main fatty acid of pomegranate seed oil. It has been previously reported that punicic acid decreases hepatic cholesterol and triglycerides, has antioxidant and tumor suppressor activities and other beneficial biologic effects[1,2]. Pomegranate is typically in season from late September to January, hence pomegranate seed oil production from fresh seeds restricts to a limited period of year. In a recent study we showed that storage of pomegranate seed oil in room temperature for one year reduces its punicic acid content from 92% of total CLA's of the oil to only 37% resulting in a major decrease in its beneficial biological activities.

The aim of this study was to investigate the CLA's pattern of pomegranate seed oil prepared from pomegranate seeds stored in room temperature for one year by ^{1}H and ^{13}C NMR spectroscopic techniques.

^{1}H NMR and ^{13}C spectra of pomegranate seed oil prepared from old seeds were completely identical with the oil prepared from fresh seeds. Punicic acid, α-eleostearic acid and catalpic acid levels in pomegranate seed oil were 92%, 5.5% and 2.5% respectively and β-eleostearic acid (trans, trans, trans) isomer was not detectable in the oil.

The results of this study have potential importance for pomegranate seed oil production and indicate that the oil prepared from old seeds have similar quality regarding CLA's with the oil prepared from fresh seeds.

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Drosophila lipidomics: Tissue specificity, the effect of diet and developmental stage

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Lipids are a diverse class of molecules that play fundamental roles in many biological processes, including the formation of cell membranes, energy storage, and signaling. Multicellular organisms produce thousands of lipids that distribute asymmetrically throughout different cell types and tissues. Although this segregation is thought to be pivotal for each tissue structure and function there has been no comprehensive lipidomic study on a single organism.

Here we report on the systematic characterization of the temporal and spatial distribution of lipids in a complex organism, *Drosophila*. By using quantitative shotgun profiling by high resolution mass spectrometry, we traced the absolute content (mol) of 250 lipid species of 14 major lipid classes in different conditions. We analyzed 27 developmental stages from egg hatching to adulthood, the 6 main tissues from larvae fed on 4 distinct diets, we covered 54 different biological conditions, from which 222 lipid extracts were prepared and more than 1300 spectra were acquired. The full lipidome analysis required less than 2 nmol of total lipid material. Spectra were acquired in three technical replicas within less than 10 minutes.

Our data show that *Drosophila* tissues greatly differ in their polar and neutral lipid content that could be readily correlated to their specific structure and function. Surprisingly, tissues exhibit different degrees of lipidome plasticity in response to dietary conditions, and differently regulate their sterol content. Furthermore, unexpected metabolic shifts emerged during larval and pupal development. Altogether, our data suggest a wide range of mechanisms by which changes in tissue lipidomes accompany developmental changes or different nutritional conditions. This study provides a comprehensive, quantitative and expandable resource for further pharmacological and genetic studies of metabolic disorders and molecular mechanisms underlying dietary response.

Vitamin E modulates the oxidant–antioxidant imbalance during cigarette smoke induced oxidative stress in rats

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Cigarette smoking is one of the major causes of mortality involving respiratory and cardiovascular illness in developing countries. Cigarette smoking is known to contain abundant of free radicals, which is able to cause tissue oxidative damage at various levels. The body has the ability to produce antioxidants. When there are excessive free radicals generated due to smoking, the available tissue antioxidants may become depleted, leading to oxidative stress. Male albino rats were induced with cigarette smoke for 70 days to assess the induced oxidative damage due to lung muscle atrophy. An enhanced lipid per-oxidation was recorded with elevated activity levels of conjugated diens, malondialdehyde and other thiobarbituric acid reactive substances (TBARS) in denervated muscle. The activity levels of antioxidant defense enzymes, viz. superoxide dismutase (SOD), catalase (CAT), glutathioneperoxidase (selenium, non selenium) (GPx), glutathione reductase (GR), glutathione-s-transferase (GST) were depleted in the cigarette smoke induced lung muscle. Vitamin E is a major antioxidant. When the Experimental control animal (cigarette smoke induced muscle) was supplemented with vitamin E, revealed a depleted lipid peroxidation and increased activity levels of antioxidant defense enzymes. In this study we investigate the efficacy of antioxidant, the supplementation of vitamin E could prevent the oxidative damage in the lung muscle despite smoking.

Discovery of lysophospholipid acyltransferases in the Lands' cycle

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Cellular membranes contain several classes of glycerophospholipids, which have numerous structural and functional roles in cells. Membrane diversity and asymmetry are important for membrane fluidity, curvature, and storage of lipid mediator precursors. Using acyl-CoAs, glycerophospholipids are first formed in the *de novo* pathway (Kennedy pathway), and then modified in the remodeling pathway (Lands' cycle) to generate mature membrane. Recently, several lysophospholipid acyltransferases (LPLATs) from two families, the 1-acylglycerol-3-phosphate O-acyltransferase (AGPAT) family and the membrane bound O-acyltransferase (MBOAT) family, were shown to function in the remodeling pathway. Until now, 10 LPLATs were identified from the two families. Among them, we discovered 6 LPLATs. First, we identified (i) lysophosphatidylcholine acyltransferase 1 (LPCAT1)[1] involved in pulmonary surfactant and platelet-activating factor (PAF) production in the lung, and (ii) LPCAT2[2], which catalyzes PAF and PC production in inflammatory cells. LPCAT2 is phosphorylated and activated by endotoxin-stimulation in macrophages. The phosphorylation site of Ser[34] was identified[3]. We also identified (iii) lysophosphatidic acid acyltransferase 3 (LPAAT3) as a testis specific acyltransferase (LPAAT and lysophosphatidylinositol acyltransferase activity)[4]. These enzymes were discovered from the AGPAT family. We also discovered several (iv-vi) LPLATs (LPCAT3, LPCAT4, and lysophosphatidylethanolamine acyltransferase 1) from the MBOAT family to generate membrane diversity[5]. Recent findings from other groups and ours have demonstrated that the two families contain LPLAT activities functioning in the remodeling pathway. Thus, many LPLATs have been identified, resulting in the greatest advance in the LPLAT field since the discovery of the Lands' cycle 50 years ago.

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Mycolic acids as diagnostic markers for tuberculosis case detection in humans and drug efficacy in mice

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Mycolic acids are attractive diagnostic markers for tuberculosis (TB) infection because they are bacteria derived, contain information about bacterial species, modulate host–pathogen interactions and are chemically inert. Here, we present a novel approach based on mass spectrometry. Quantification of specific precursor-fragment transitions of approximately 2000 individual mycolic acids (MAs) resulted in high-analytical sensitivity and specificity. We next used this tool in a retrospective case-control study of patients with pulmonary TB with varying disease burdens from South Korea, Vietnam, Uganda and South Africa. MAs were extracted from small volume sputum (200 μl) and analyzed without the requirement for derivatization. Infected patients (70, 19 of whom were HIV positive) could be separated from controls (40, 20 of whom were HIV positive) with a sensitivity and specificity of 94 and 93%, respectively. Furthermore, we quantified MA species in lung tissue of TB-infected mice and demonstrated effective clearance of MA levels following curative rifampicin treatment. Thus, our results demonstrate for the first time the feasibility and clinical relevance of direct detection of mycobacterial lipids as biomarkers of TB infection.

Polar lipid derangements in type 2 diabetes Mellitus

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The homeostatic balance and precise partitioning of lipids within the body is thought to be intricately linked to the pathophysiology of Type 2 Diabetes Mellitus (T2DM). Herein, we report a comprehensive lipidomic profiling of the plasma of T2DM patients of three distinct levels of severity, in comparison to healthy volunteers adjusted for BMI. Total cholesterols (Cho) and cholesteryl esters (CE) were significantly higher in Grade 1 ($p = 0.07$ for Cho; $p < 0.001$ for CE) and Grade 2 patients ($p < 0.01$ for Cho; $p < 0.001$ for CE); but these aberrant alterations in neutral lipid levels were not observed in Grade 3 insulin-treated patients. Notably, it was found that individual GM3 species displayed a consistent trend across the four groups of subjects, which was dependent on the chain length of their fatty acid moieties attached. Interestingly, scatter plots of individual GM3 species showed that majority of the GM3 species that differed appreciably between different grades of T2DM patients and controls were also significantly correlated with clinical indicators of the disease, such as fasting blood glucose (FBG) and homeostasis model assessment 2 of steady state b-cell function (HOMA2-%b). These findings suggest that GM3 species could be of substantial significance in mediating insulin resistance in a manner that is dependent on their acyl lengths. In addition, reduction in phosphatidylcholines (PC) and elevated levels of phosphatidylethanolamines (PE) in Grades 1 and 2 patients resulted in a drop in PC:PE ratio in T2DM, which was not present in the insulin-treated patients. The altered ratio of PC:PE might be implicated in the increased level of lipogenesis observed in T2DM. The positive correlation of total lysophosphatidylinositols (LPI) with HOMA2-%b; and the significant reduction in total LPI with an accompanying decrease in HOMA2-%b observed in the current study further support the previously proposed insulinotropic roles of LPI. Also for the first time, we report altered levels of the unusual phospholipid, lysobiphosphatidic acid (LBPA), in the plasma of diabetic patients. Notably, species of LBPA and LPI represent the only lipid classes for which the abnormalities were similarly observed in insulin-treated patients.

A Novel Tool to Study *Mycobacteria* Dormancy

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Mycobacteria, like some other prokaryotic species, are able to accumulate large amounts of neutral lipid forming structures called lipid bodies (LB) in their cytoplasm. These LBs are probably involved in the bacteria cell life cycle, as recent reports have shown that tubercular bacilli in lung granulomas are enriched in LB. These TG deposits are consumed when hypoxic dormant bacilli are reactivated, suggesting that lipid storage may contribute to mycobacterial survival in a non-replicating state during the latent and/or reactivation phase. In fact, the TG degradation process catalyzed by lipolytic enzymes may release free fatty acids, which can be utilized as a carbon source during growth and infection processes. Several studies have explored the possibility of inhibitors targeting these lipid-catabolizing enzymes as a better target towards latent tuberculosis, with little success.

Our group has previously shown that tetrahydrolipstatin (THL), an irreversible inhibitor of serine esterases, inhibits mycobacterial lipases by preventing the breakdown of TG to fatty acids. Despite THL being considered a superior anti-mycobacterial drug, its mechanism of action is still controversial, with different groups claiming different proteins as THL targets. To better understand THL activity and its biological role, we used a novel chemical proteomic strategy to look for specific mycobacterial target and validate its biological activity by metabolic profile study. We synthesized membrane penetrable THL analogues differing in position of alkyne handle. The protein bound THL-analogues are then visualized /or enriched by 'click-reaction' (Huisgen cycloaddition reaction) with azide-handle fluorescent dye or biotin tag. We report that THL treatment changes the storage and membrane lipidome of tubercle bacilli, by inhibiting lipases in dormant bacilli and replicative phase of bacterial growth.

Comparative mycolic acid profiling of *Mycobacterium tuberculosis* lineages: A correlation to virulence

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Keywords: Mycolic acids, *Mycobacterium tuberculosis*, Lipidomics

Mycolic acids (MA) are alpha alkyl, beta hydroxyl long chain fatty acids found exclusively in the cell wall of bacteria belonging to the *Mycobacterium*, *Rhodococcus*, *Nocardia*, and *Corynebacterium* genera. MA provides protection against dehydration, hydrophobic antibiotics and hostile environment of macrophage thereby playing a crucial role in the survival of the bacterium. Drugs that are used to treat Tuberculosis caused by *Mycobacterium tuberculosis* (Mtb) are targeted at the mycolic acid biosynthetic machinery. Intensive research is still in progress in search of efficient drugs to combat the emerging multi drug resistant species (MDR) and extremely drug resistant species (XDR). The comparative MA profiling would enable the correlation of fine structure of MA to virulence. Lipidomics is a powerful tool for comparative lipid profiling in different biological systems. Using lipidomics approach we have systematically analyzed the MA from various Mtb strains that have been classified into lineages. MA being a prominent cell wall lipid and key virulence factor in Mtb, its interaction with the cellular immune response is of significant interest. Hence, the dynamic fluctuations of the MA composition in a host environment have also been studied by profiling MA extracted from Mtb infected macrophage.

Lipidomics of diverse microalgae by electrospray mass spectrometry

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Glycerolipids, lipids made of a glycerol backbone esterified with fatty acids, play an important role in metabolic processes such as energy storage or cell integrity. Among these lipids, the neutral triacylglycerols (TAGs), used by the cell mostly for energy storage, constitute an important area of investigation due to their potential use as biodiesels feedstock. In microalgae, TAGs are accumulated to high levels within the cell under stress conditions such as in the stationary growth phase or in nutrient starvation. TAGs from microalgae have many other applications. For instance, while low polyunsaturated fatty acid (PUFA)-containing TAG are good for biodiesel, the high PUFA-containing TAG can be used in food supplements or pharmaceutical applications. Although microalgal lipids are highly valuable, the understanding of lipid metabolism pathways in microalgae is currently limited. Hence, more in-depth analysis of the lipidome of these organisms is needed to help elucidate their lipid metabolism. Beyond TAG production, analysis of polar glycerolipids including phospholipids, galactolipids and sulfolipids, amongst others, is of interest. As part of a collaborative project, the 1K plant transcriptome project (http://www.onekp.com), we address some of these limitations by undertaking lipidomics analysis of 27 algae strains (including *Chlamydomonas reinhardtii*). Herein is described our comprehensive analytical workflow which makes use of shotgun as well as on-line chromatographic (normal and reverse phase) approaches and a array of mass spectrometric techniques including single-stage, tandem and high resolution scans. Significant differences were observed in the lipid contents of the studied microalgae.

A lipidomics study of two putative lysophosphatidic acid acyltransferases in *Saccharomyces cerevisiae*-Ydr125cp (Ecm18p) and Ydro18cp

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All eukaryotes utilize glycerophospholipids to build their membranes and stock triacylglycerols as energy reserve when nutrition is abundant. Both lipid classes are made from phosphatidic acid (PA), a key intermediate in the biosynthesis. During the *de novo* synthesis of phospholipids, acyl chains are incorporated into the *sn*-2 position of lysoPA to generate, by 1-acyl-*sn*-glycerol-3-phosphate acyltransferase (AGPAT), also known as lysophosphatidic acid acyltransferase (LPAAT). By means of Integrated Weighted Averaging (IWA), a scalable and efficient function prediction framework, two yeast proteins (Ydr125cp and Ydro18cp) were predicted with a new function—LPAAT activity. In this study, we aim to verify the prediction of these two genes respectively with mass spectrometry analysis. The identification and characterization of LPAAT will provide more comprehensive insights on gene network involved in phospholipid biosynthesis and remodeling, which is essential for membrane integrity and cell viability.

Phosphoinositides analysis by mass spectrometry

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Phosphoinositides are important lipid mediators of signalling events in all cellular compartments. They play a key role in the attachment of the cytoskeleton to the plasma membrane, exocytosis, endocytosis, membrane trafficking and the activation of enzymes. The analysis and quantification of biologically important lipids became a routine process in lipidomics, however the phosphoinositides analysis has not progressed at the same rate and it is still a challenging research field. This is due to their low cellular concentration, their highly charged head groups that affects extraction and detection and their presence as a complex with phosphoinositide binding proteins. In this work, new approaches to examine phosphoinositides in biological samples by nanoLC-ESI-MSMS are presented.

Effects of lipids in neural stem cells proliferation and differentiation

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Neural stem cells (NSCs), present during embryonic development and in certain regions of the adult central nervous system (CNS), can self-renew, proliferate, and differentiate into neurons or glial cells, which provides a reservoir for replacement of cells lost during normal cell turnover and after brain injury. Therefore, NSCs have been postulated as potential treatment of human neurodegenerative disorders. However, prior to using NSCs, factors controlling the proliferation and differentiation of NSCs need to be identified. In recent years, lipids have been found as active molecules in many biological processes; thus, in this study, we try to elucidate the effect of lipids in NSCs proliferation and differentiation.

Our preliminary data showed that during the growth of neurospheres, the amount of several lipids changed consistently, with increasing numbers of NSCs. Changes in neurosphere lipid composition implied a possible involvement of these lipids, such as 7-hydroxycholesterol, 7-keto-cholesterol and sphingomyelin, in the proliferation and differentiation of NSCs. The future direction of our study will focus on verifying the effect of lipid candidates involved in the process, by introducing lipid treatment into NSCs proliferation and differentiation, and further revealing the underlying mechanisms.

Effects of temperature stress on membrane lipidome composition of *Saccharomyces cerevisiae*

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Lipids are recognized as important structural molecules, storage fuels, as well as functional metabolites. Different classes of lipids, varied in their head groups, backbones, acyl and sphingoid base chain lengths, and the degrees of saturation and hydroxylation, are essential components of the architectures of every single cell. Hence, the homeostasis of lipids is closely related to the robustness of the cell. Using combined lipidomics methods, we were able to characterize the lipidome of Saccharomyces cerevisiae cultured in different temperatures and detect the expression level of 168 molecular lipid species. Our preliminary results showed that the lipidprofile pattern of wild type yeast (W303) was changed under temperature stress. These profile data would provide the basic foundation for the proposal of the yeast's remodeling of the lipid composition to cope with the temperature stress and the further biochemical and genetical validation.

The lipidomics study of *Magnaporthe* suggests a possible mechanism of turgor pressure production

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Magnaporthe oryzae (*M. oryzae*) is the causal agent of the rice blast disease and breaks down triacylglycerides (TAGs) to generate turgor pressure as a mean for breaking into host's leaf cells. Lipids therefore play a very important role in the pathology. Here we have systematically profiled major lipid components found in *M. oryzae* using a biochemical lipidomic approach. This time resolved atlas should form a hypothesis generating basis for functional assessment of lipid metabolism during the *M. oryzae* life cycle.

Molecular mimicry between gangliosides and bacterial lipo-oligosaccharides can cause human autoimmune disease

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Molecular mimicry between self and microbial components has been proposed as the pathogenic mechanism of autoimmune diseases and this hypothesis is proven in Guillain-Barré syndrome. Guillain-Barré syndrome, the most frequent cause of acute neuromuscular paralysis, sometimes occurs after *Campylobacter jejuni* enteritis. Gangliosides are predominantly cell-surface glycolipids highly expressed in nervous tissue, whilst lipo-oligosaccharides are major components of the Gram-negative bacterium *C. jejuni* outer membrane. IgG autoantibodies to GM1 ganglioside were found in the sera from patients with Guillain-Barré syndrome. Molecular mimicry was demonstrated between GM1 and lipo-oligosaccharide of *C. jejuni* isolated from the patients.

Disease models by sensitization of rabbits with GM1 and *C. jejuni* lipo-oligosaccharide were established. Guillain-Barré syndrome provided the first verification that an autoimmune disease is triggered by molecular mimicry. Its disease models are helpful to further understand the molecular pathogenesis as well as to develop new treatments in Guillain-Barré syndrome.

Lipidomics Establishes Meibum as the Primary Source of Tear Film Lipids

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Identification of the complete lipidome of the human tear film may be vital in improving the understanding of the pathology of dry eye disease. The primary source of lipids in human tear film is believed to be meibum, a waxy secretion of the meibomian glands. Lipidomic analysis of human tears and meibum present a number of unique challenges to the analytical chemist. The small sample size and low lipid concentration of human tears requires specialized sample handling, while the predominance of hydrophobic lipids within the tear and meibum lipidomes pushes the limits of electrospray ionization mass spectrometry: presenting unique challenges in overcoming ion suppression. In addition, novel and rare lipid classes are found in tears, requiring both specialized mass spectrometric methods and internal standards.

In order to overcome these challenges, we have developed specialized extraction and data collection techniques. Lipids were extracted by bi-phasic methods utilizing methyl tert-butyl ether, and the extracts analyzed using an AB Sciex QTRAP 5500 equipped with an Advion Nanomate chip-based nano-electrospray source. To quantify the new lipid classes found in tears, authentic internal standards have been synthesized. Targeted precursor ion and neutral loss scans were used to identify and quantify 185 individual lipid species in human tears and meibum.

Quantification of six lipid classes, including (O-acyl)-omega-hydroxy fatty acids, cholesterol esters, wax esters, triacylglycerols, phosphatidylcholines and sphingomyelins was performed. Our pilot study compares the lipidome of human tears and meibum, and suggests that the primary source of tear film lipid is indeed meibum. Comparison between patient-matched meibum and tear samples demonstrates that although disparities exist between patients, matched meibum and tears have similar lipid class profiles. In addition, within each lipid class the profiles of individual species were found to be strikingly similar. Day-to-day lipid profile variations were found to be minor. Interestingly, phospholipids were found at substantially higher levels in tears. Further data will be presented analyzing different meibum collection techniques and the effects on lipid profiles. This work demonstrates the similarities in lipid profiles between tears and meibum, and in addition highlights the analytical techniques that have allowed us to investigate these challenging samples.

Molecular Genetics and Quantitative Proteomics to Identify Novel Regulators of Lipid Metabolism

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Stable isotope labeling by amino acids combined with mass spectrometry is a widely used methodology to quantitatively examine metabolic and signaling pathways in yeast, fruit flies, plants, cell cultures and mice. We have recently shown that C. elegans can be completely labeled with heavy-labeled lysine by feeding worms on prelabeled lysine auxotroph Escherichia coli for just one generation. We applied this methodology to examine the organismal response to functional loss or RNAi mediated knock down of various transcription factors and kinases regulating lipid metabolism and have identified a number of novel regulatory components of lipid metabolism. Moreover, combined with site-specific proteomics and lipidomics we have pinpointed specific posttranslational modifications of specific proteins that are important for their specific function in lipid metabolism and cellular energy homeostasis.

Top-down lipidomics and risk of developing cardiovascular disease

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Objective

The goals of this study were to test (1) whether characteristic changes of the plasma lipidome predicts development of cardiovascular disease (CVD) and (2) whether variation of the Phosphatidic acid phosphatase type 2B (PPAP2B) gene (rs17114036), which is implicated in lipid metabolism and was recently strongly associated with coronary artery disease (CAD) in the CARDIOGRAM study, affects CVD associated lipid species.

Design and method

High throughput screening of the fasted plasma lipidome was performed by mass spectrometry in 211 individuals who developed CVD during 12 years of follow-up and in 216 controls from the cardiovascular cohort of the prospective population-based Malmö Diet and Cancer Study (MDCS-CC). Plasma lipids were extracted using a liquid handling station and identified and quantified by shotgun analysis on a LTQ orbitrap using the LipidXplorer software.

Results

Each s.d increase of baseline levels of 2 different lysophosphatidylcholines (LPC), LPC16_0 (OR = 0.79; 95% CI 0.65–0.97; P = 0.028) and LPC20_4 (OR = 0.77; 95% CI 0.61–0.96; P = 0.024) were associated with a decreased risk for CVD after adjusting for Framingham risk factors. Sphingomyelin (SM) 38_2 was associated with increased odds of future CVD (OR = 1.28; 95% CI 0.99–1.64, P = 0.057). Five triglyceride (TAG) species were protective (OR = 0.78–0.82, P = 0.031–0.049). LPC16_0 was negatively correlated with the intima media thickness of the common carotid artery (P = 0.010) and with plasma CRP level (P = 0.002) whereas SM38_2 was positively correlated with CRP (P = 0.008). Carriers of the CAD-associated PPAP2B allele had reduced levels of multiple phosphatidylcholine and phosphatidylcholine-ether species (P = 0.001–0.053) as well as of LPC16_0 (P = 0.056).

Conclusions

Our data suggests that CVD development is preceded by reduced levels of LPC16_0, LPC20_4 and several TAG species as well as by increased levels of SM38_2. Part of the relationship between decreased LPC16_0 and CVD risk may be mediated by genetic variation of the PPAP2B.

A low ratio of n-6/n-3 PUFA promotes the formation of anti-inflammatory mediators in macrophages

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Introduction

The presence of a chronic subclinical inflammatory state in adipose tissue and adipose tissue macrophages is a hallmark of obesity and plays a key role in the development of obesity-associated complications such as insulin resistance, artherosclerosis, type 2 diabetes and non-alcoholic fatty liver disease.Lipid mediators derived from omega-3 (n-3) polyunsaturated fatty acids (PUFAs) have been implicated in the modulation of macrophage function and were shown to reduce secretion of pro-inflammatory cytokines, while omega-6 PUFA metabolites are mostly pro-inflammatory. This could be due to the formation of anti-inflammatory and pro-resolving lipid mediators such as resolvins from n-3 PUFA.

Methods

We tinvestigated the formation of lipid metabolites and mediators in a human monocyte/macrophage cell line (THP-1) after PMA induced differentiation and treatment with a high n-6/n-3 PUFA-ratio (20/1) versus a low n-6/n-3 PUFA ratio (1/1).

Results

We show that a 1/1 ratio of n-6 (arachidonic acid, AA) / n-3 (eicosapentaenoic acids, EPA and docosahexaenoic acid, DHA) PUFAs results in significantly increased formation of the pro-resolution and anti-inflammatory metabolites 18-hydroxyeicosapentaenoic acid (18-HEPE), 17-hydroxydocosahexaenoic acid (17-HDHA) and 14-hydroxydocosahexaenoic acid (14-HDHA) as compared to the 20/1 ratio. Moreover, THP-1 cells treated with the 1/1 PUFA ratio release significantly lower levels of PGE2 and particularly PGD2 as compared to the 20/1 PUFA treated cells.

Conclusions

These results suggest that a low ratio of n-6/n-3 PUFA is promoting the formation of pro-resolving and anti-inflammatory hydroxylated mediators from n-3 PUFA in macrophages. The reduced PGE2 and PGD2 levels reinforce the anti-inflammatory effect of this intervention. A balanced n-6/n-3 PUFA ratio thereby contributes to a shift towards anti-inflammatory lipid mediators away from the pro-inflammatory n-6 PUFA derived prostaglandins.

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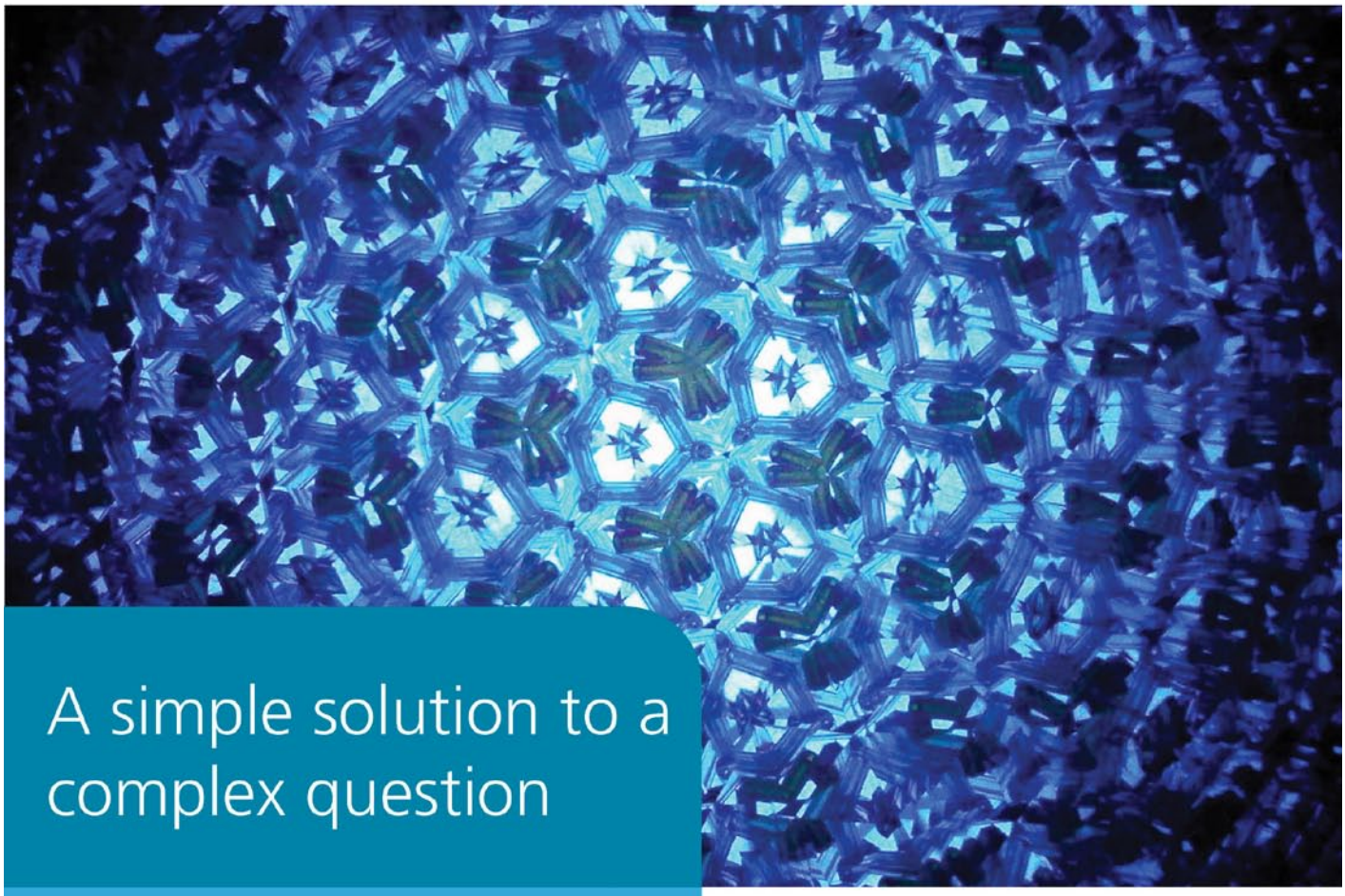

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