Interview

Lipidomics is going great guns: Interview with Kai Simons about the power of shotgun lipidomics

Lucie Kalvodova and Kai Simons

There are thousands of lipid species in living cells but we do not fully understand this diversity as it is not possible to pin down a specific function to each lipid species. Lipids have structural, metabolic and signaling functions. While signaling lipids can be understood and functionally characterized in a way similar to proteins, membrane lipids act collectively. This may have been one of the reasons for lipid neglect in the past. In order to understand the complexity of lipids in membranes, lipoproteins, body fluids, and organisms in general, we need to know the big picture which can only be delivered by modern lipidomic analyses. This has been increasingly appreciated mainly in the biomedical area. It is likely that interpretation of blood cell lipidomes will lead to the discovery of new biomarkers and lipid signatures of pathological states and cardiovascular disease risk, and so the era of HDL/LDL, TAG and cholesterol quantification may soon be over. But that is just one example. The motto of this year's Euro Fed Lipid Congress reads "From lipidomics to industrial innovation". Indeed with the advancement of mass-spectrometry and computational approaches to analyze and understand big data, lipidomics is becoming more interesting for clinical applications and industry.

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LK: Your work has made a deep impact in cell biology where you are known as the inventor of lipid rafts and master of cell membranes. We should not start discussing rafts here in detail because that would explode our page budget. Let's talk about lipidomics. If I remember correctly, your first "real", modern MS-based lipidomics project was the yeast lipidome. Why yeast?

KS: Yeast is the simplest eukaryotic organism and also its lipidome is less complex than that of the mammalian cells, especially for the glycolipids. Thus it was possible to have internal standards for all lipid classes to allow precise quantification. We had to produce the glycolipid standards ourselves. But the motivation for the work on yeast was of course connected to lipid rafts: we were trying to characterize how lipids were sorted into secretory vesicles during assembly in the trans-Golgi complex. So the driving force for me was to develop lipidomics technology to analyze lipid rafts in cell membranes and to understand how vesicular trafficking generates the lipid compositions in different cell membranes. The methods available for lipid analysis at that time were not able to answer these questions, and so new lipid approaches were desperately needed.

LK: The research progress in lipidomics is largely dependent on the technology. In the 1960s, lipids were studied by thin layer chromatography, later by GC/MS, HPLC, and modern lipidomics based on MS emerged in 1990s. How "good" are the current MS approaches and what are their limitations? There is a vast diversity of lipids in living cells (which we still don't fully understand)—the number of lipid species in lipidomes is in the order of thousands. What is the coverage of the current MS based lipidomic approaches?

KS: Together with Andrej Shevchenko, we have developed the shotgun lipidomics approach. It is different from LC/MS in that the whole sample is injected directly into the machine without pre-separation. It is fast and most importantly it is quantitative. It has great coverage: in human plasma we can analyse 19 lipid classes and more than 400 lipid species. In LC you first separate the lipids and this requires more time and is more expensive. LC has the problem that the standards start to separate from the lipids that you want to quantitate, and this can compromise quantification. There are thousands of lipids and most of them will be detected close to the noise level. This is in fact why the widest possible coverage might not always be desirable because it makes the interpretation difficult, you get false positives etc.

LK: However, low abundance lipids could also be very important—while most of the membrane lipids act collectively, "bioactive" lipids including for example signaling molecules such as eicosanoids act at low concentrations.

KS: Yes of course, these two lipid categories have to be analysed separately. Bioactive lipids require LC/MS. For membrane lipids, the analysis has to comprise a comprehensive lipidome but most of the low abundance lipids do not need to be included. We want to understand how changes in lipid composition are compensated for; the bilayer is in contrast to the aqueous space limited to two dimensions. The membrane lipids function as collectives. Here is where the lipid rafts come into the picture. Essentially we need quantitative results so that data from different labs can be compared. Quantification is a major problem with all current *omics* technologies. They are often not quantitative in absolute terms.

LK: Does lipidomics in your view have more potential for use in disease diagnostics than for example proteomic technologies? Why is lipidomics so underrepresented in the current applied and clinical research?

KS: Yes, I consider that lipidomics meets all the requirements because we can get absolutely quantitative results. This is important in basic research but it is also the reason why lipidomics is now becoming an excellent tool in clinical and applied research. The food and nutraceutical industry needs objective data to provide evidence that supplements work, and lipidomics can provide such data in a much more convincing way than relative comparisons with all their limitations. One issue is a lack of coherence, the results cannot be compared between labs as already mentioned. In clinical diagnostics for identification of validated multiparameter biomarkers, only quantitative signatures will do.

LK: Almost every institute has a proteomics facility but lipidomics core labs are rare. Why is that?

KS: Lipid research and lipidomics are areas that have been neglected due to methodological difficulties. Therefore few scientists have been trained in these areas. Scientists who have protein training abound. Therefore, the know-how is lacking to build up lipidomics core facilities. Simply buying the mass spectrometers and then believing that you can then proceed to provide lipidomic data is wishful thinking. So many essential elements will still be missing.

LK: Which areas would you like to focus on most in the future? Translating the shotgun lipidomics technology you have developed into clinical applications?

KS: Yes, clinical applications of lipidomics are for me a major challenge and focus. That is why I have now started a spin-off company because at a large research institute such as MPI-CBG there are too many users who change the machine settings all the time—reproducibility is always a problem—and there are also issues with capacity. Introducing plasma, serum and blood cells lipidomics into clinical diagnostics is one of the major challenges for the future. Erythrocytes have only one membrane, which makes them easy to work with, and we already know that their membranes reflect the dietary status, at least as far as omega-3s are concerned. But we need a deeper understanding of how well erythrocytes mirror the clinical status of the patients. As we discussed before, lipids behave collectively. What this means for defining deviations from the norm has to be explored. Simply put, we need to understand what a healthy and disease-specific lipidome is. We want to define lipotypes for health and disease.

The shotgun methodology is so robust, comprehensive and quantitative that it can be taken to the clinic, into food R&D and into other applications. In addition it is a fast and high-throughput technology—in 15 minutes you have the whole lipidome.

LK: Thanks Kai for taking the time to talk to us.

Lucie Kalvodova, Managing Editor, EJLST and former PhD student of Kai

Kai Simons is Emeritus Director of the Max Planck Institute for Molecular Cell Biology and Genetics and CEO of Lipotype GmbH in Dresden. His recent research has focused on cell membrane organization and function. He has pioneered the concept of lipid rafts as a membrane organizing principle, based on the phase-separating capabilities of sphingolipids and cholesterol in cell membranes. For his contributions to cell biology, Kai has received numerous accolades, including the Keith Porter Lecturer of the American Society for Cell Biology, a Harvey Society Lecturer, Dunham Lecturer at Harvard Medical School, and Li Lecturer, UC Berkeley. He has received the Anders Jahre Prize for Medical Research, the Runeberg Prize, Finland, the Laurens van Deenen Medal, University of Utrecht, the Schleiden Medal of the Academy Leopoldina and the Äyräpää Prize, Finland. He is Doctor Honoris Causa at the Universities of Geneva, Oulu and Kuopio, Finland and Leuven, Belgium. Kai is a foreign member of the National Academy of Sciences, USA, and was the President of the European Life Scientist Organization. In 2007–2008 Kai was co-director of the Shanghai Institute for Advanced Studies of the Chinese Academy of Science. With Lipotype, 2013 winner of the start-up competition *futuresax* of the Free State of Saxony, Kai is now focusing on translating lipidomics to clinical and industrial applications.

12th EuroFedLipid Congress (14–17 Sep 2014, Monpellier)

Lipidomics, Analytics, Authenticity, Imaging session program

Chair: Anna Nicolaou

Keynote Lecture
Natural Variation of Lipidomes
M. Wenk, Singapore/SG

Characterization of Retina's Ganglioside Profile by Liquid Chromatography Coupled to Mass Spectrometry
E. Sibille, Dijon/FR, E. Masson, L. Martine, F. Picquet, O. Berdeaux, Dijon/FR

Comprehensive High Throughput Serum Lipidomics by Shotgun MS
J.L. Sampaio, Dresden/DE, C. Fernandez, Malmo/SE, M. Surma, R. Herzog, C. Klose, C. Lauber, A. Vasilj, L. Kaderali, Dresden/DE, O. Melander, Malmo/SE, K. Simons, Dresden/DE

A Unique Brain Lipidome and Metabolome Biosignature in Alzheimer's Disease
G. Astarita, Milford/US, G. Paglia, Reykjavik/IS, L. Steven, Milford/US, L. James, Manchester/GB

A Novel Lipidomic Platform to Determinate Lipid Mediators Associated to the Healthy effect of Marine Lipids in Metabolic Syndrome
M.I. Medina, Vigo-Pontevedra/ES, M. Pazos, Vigo-Pontevedra/ES, J.M. Gallardo, Vigo-Pontevedra/ES, I. Rodriguez, Santiago de Compostela/ES, R. Cela, Santiago de Compostela/ES, G. Dasilva Alonso, Vigo-Pontevedra/ES

Elevation of Lipid Peroxidation in Maternal-Fetal Mice Exposed to Industrial Contaminant Perfluorooctane Sulfonate
J.C.Y Lee, Hong Kong/HK, Y.Y. Lee, Hong Kong/HK, C. Oger, J.M. Galano, T. Durand, Montpellier/FR, C.K.C. Wong, Hong Kong/HK

The Role of NMR in the Screening of Edible Oils using Targeted and Non-targeted Methods
M. Link, Rheinstetten/DE, M. Spraul, H. Schaefer, F. Fang, E. Humpfer, Rheinstetten/DE

Ultrasound Doppler based in-line Viscosity and Solid Fat Profile Measurement of Fat Blends and Emulsions
K. Bhattacharya, Brabrand/DK, N. Young, Brabrand/DK

Analysis of Oil – Biodiesel Samples by HPLC using the Normal Phase Column of New Generation and the Evaporative Light Scattering Detector
S.N. Fedosov, Aarhus/DK, N.A. Fernandes, Lages/BR, M.Y. Firdaus, Aarhus/DK

