Beutenberg Campus /IMPRS-gBGC Workshop

Method and Concept Transfer

9th June 2011 13:00 – onwards

Lecture Hall, Abbe Center Beutenberg

Beutenberg Campus e.V.

International Max Planck Research School for

Global Biogeochemical Cycles

The aim of this workshop is to inspire interdisciplinary cooperation and networking. The presentations can either have a **methodological** or a **conceptual approach**. The idea is to establish contacts between scientists with complementary skills and needs.

Organisers: Dr. Susanne Erland, Beutenberg Campus & Anna Görner, IMPRS-gBGC

PROGRAM :

13:00 **Prof. Dr. Hartmut Bartelt** (Chairman of Beutenberg Campus Jena e.V.)
WELCOME ADDRESS and Chair of sessions

Session 1:

13:10 **Dr. Valérie Schwab-Lavrič** (MPI-BGC)
Purification of molecular compounds for subsequent isotopic analysis using high-performance liquid chromatography–mass spectrometry

13:35 **Dr. Torsten Frosch** (IPHT)
Gassensing and Fiber sensors

14:00 **Dr. Benjamin Dietzek** (IPHT) *Campus Award Winner 2011*
Molecules in motion - How dynamics lead to function

14:25 *Coffee Break*

Session 2:

14:45 **Dr. Nils Hartmann** (FLI)
The short-lived fish Nothobranchius furzeri as a model for age research

15:10 **Dr. Hanna Heidel-Fischer** (MPI-CE)
Old genes meet new technologies: Revealing the evolution of butterfly genes via selected sequencing approaches

Session 3:

15.35 **POSTERS** *with Refreshments*

Purification of molecular compounds for subsequent isotopic analysis using high-performance liquid chromatography–mass spectrometry

Valérie F. Schwab-Lavrič

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Since the first gas chromatograph–isotope ratio mass spectrometer systems (GC–IRMS) became commercially available, compound-specific isotope analyses (CSIA) have proven to be a valuable tool in environmental researches [e.g. 1;2]. However, such studies are often hampered by the difficulty to separated GC-amenable compounds (e.g., terpenoids, sterols, hopanols) with similar molecular weight, polarity and volatility which results in densely populated gas chromatograms that are not suitable for analysis by GC–IRMS requiring baseline separation of analytes. Consequently, compounds must have been substantially pre-purified prior to GC–IRMS analysis. Purifications, typically involving amongst others multiple chromatographic urea adduction and column or thin-layer chromatography with AgNO3-adducted silica gel, are often complicated, time consuming and difficult to perform in a reproducible manner. Results are often not satisfactory, with low recoveries and the potential for isotope fractionation during workup.

High-performance liquid chromatography (HPLC) provides a substantially higher resolution than column or thin-layer chromatography. Separations can be performed in a reproducible manner and sample injection can be automated. HPLC coupled to mass spectrometer and fraction collector allows compound collection based on mass or elution time and offers a highly valuable tool for compound purification. Here, we present methods for purifying various sterols and alkenones from sediment lipid extracts with (semi)-preparative-HPLC–MS (APCI) [3]. The methods were developed to minimize sample handling and maximize recovery so that CSIA could be performed on compound-deficient samples. We also present evidence that substantial hydrogen isotope fractionation can occur across an HPLC peak. Measurements of δD values across individual HPLC peaks of standards demonstrate the necessity of collecting at least 92% of the peak in order to maintain isotopic integrity of the purified compounds. Different applications of the methods in environmental and cell physiological researches will be presented [3;4].

[1] D. Sachse, J. Radke, G. Gleixner, Geochim. Cosmochim. Acta 68 (2004) 4877.

[2] Y. Chikaraishi, H. Naraoka, Geochim. Cosmochim. Acta 69 (2005) 3285.

[3] V.F. Schwab , J. Sachs, Organic Geochemistry 40 (2009) 111.

[4] V.F. Schwab , J. Sachs, Geochim. Cosmochim. Acta XX (2011) In revision.

Gas sensing and Fiber sensors

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Measuring the composition and variation of biogenic gasses plays an important role in the characterization of complex ecosystems. Conventional methods for gas analysis, are often based on separation of analytes by gas chromatography (GC) and successive identification and quantification e.g. by means of mass spectroscopy (MS). These devices are expensive in acquisition and operation, need sample preparation steps and offer limited degree of miniaturization and time resolution. Contrariwise, optical fiber sensors are highly miniaturized, sensitive, flexible, lightweight and applicable in harsh environment.

Fiber optical Raman sensing is a promising approach for on-site gas characterization. Advantages are the low analyte demand and effective light-guiding in the fiber, resulting in a strong light-analyte-interaction and thus a high sensitivity. Raman spectroscopy is based on inherent molecular signals and can be exploited for label-free and non-consumptive sensing. These fingerprint-like and narrow Raman signatures allow for the simultaneous analysis and quantification of several gasses and volatile compounds. Raman fiber optical gas sensing provides unique capabilities, such as fast and permanent on-site monitoring of various biogenic gasses, (N_2, O_2, CO_2, CH_4, N_2O, etc.), high temporal (monitoring, exchange rates) and spatial resolution (depth and location). This emerging technique is a valuable extension in the toolkit regarding the research of the interplay and interdependencies of physical, chemical and biological processes.

Molecules in motion - How dynamics lead to function

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The range of molecules and materials, which perform function after absorption of light, is manifold: The protein rhodopsin is involved in the light perception of mammalians, complex material blends are used as materials in organic photovoltaics and photodynamic therapy is used to treat carcinomas. Despite the wide range of photoactivated molecules and materials ultrafast excited-state processes generally lead the way to the function of the molecules. These ultrafast processes occur on the characteristic time-scale for nuclear motion in molecules, *i.e.*, on the time-scale of 100 femtoseconds (fs, 10^{-15} s) to some picoseconds (ps, 10^{-12} s).

In this presentation the experimental strategies to investigate such ultrafast molecular processes are presented and their application to study, *e.g.*, photoinduced processes in supramolecular catalysts is exemplified. The latter systems present a potentially interesting approach to convert sunlight into molecular hydrogen, which might be used as an alternative environmentally clean fuel.

The short-lived fish *Nothobranchius furzeri* as a model for age research

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Among vertebrates that can be kept in captivity the annual fish *Nothobranchius furzeri* possesses the shortest known lifespan. It also shows typical signs of ageing and is therefore an ideal model to assess the role of different physiological and environmental parameters on ageing and lifespan determination. Here we used *Nothobranchius furzeri* to study whether ageing is associated with mitochondrial DNA (mtDNA) alterations and changes of mitochondrial function. We sequenced the complete mitochondrial genome of *N. furzeri* and found an extended control region. Large-scale mtDNA deletions have been frequently described to accumulate in other organisms with age, but there was no evidence for the presence of detectable age-related mtDNA deletions in *N. furzeri*. However, mtDNA copy number significantly decreased with age in skeletal muscle, brain, liver, skin and dorsal fin. Consistent with this finding, expression of *Pgc-1α*, that encodes a transcriptional co-activator of mitochondrial biogenesis and expression of *Tfam* and *mtSsbp* both encoding mtDNA binding factors was down-regulated with age. The investigation of possible changes in mitochondrial function revealed that the content of respiratory chain complexes III and IV was reduced in skeletal muscle with age. In addition, ADP-stimulated and succinate-dependent respiration was decreased in mitochondria of old fish. These findings suggest that despite the short lifespan, ageing in *N. furzeri* is associated with a decline in mtDNA copy number, the down-regulation of mtDNA-associated genes, and an impairment of mitochondrial function.

1) Hartmann et al., Mechanisms of Ageing and Development (2009) 130, 290–296.

2) Reichwald et al., Genome Biology (2009) 10, Issue 2, Article R16

3) Hartmann et al., Aging Cell, in press

Old genes meet new technologies: Revealing the evolution of butterfly genes via selected sequencing approaches

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Over the last 30 years nearly all DNA sequencing techniques have been based on the Sanger biochemistry, first published by Fred Sanger in 1977[1]. However, in the past six years completely new sequencing strategies have emerged [2] reducing the cost of sequencing drastically and at the same time increasing the amount of data per run tremendously. These next generation sequencing techniques (NGS) are evolving rapidly, presenting researches with challenges such as how to effectively analyze the greatly increased data and how to design experiments accordingly.

In my talk I will give an overview on Sanger and NGS techniques using as an example our long standing research on the endemic butterfly Small Cabbage White (*Pieris rapae*) and its relatives. The Small Cabbage White caterpillars feed on cabbage plants and posses a unique detoxification enzyme, called nitrile-specifier protein (NSP) to overcome the plant defenses. I will explain how state of the art traditional and new sequencing techniques were productively used to identify the enzyme and track its molecular evolutionary history.

[1]Sanger F. et al. Nature, 687-695 (1977).

[2]Margulies M. et al. Nature, 376-80 (2005).