TransBio Project (13 & 14 of February 2014)

General introduction

The need for comprehensive analysis of metabolism using metabolomics or fluxomics is rapidly growing in a broad range of application fields, including health, biotechnology, nutrition and food safety, as well as for basic understanding of biological organisms. In this context, MetaToul, the Toulouse Metabolomics & Fluxomics Facilities (www.metatoul.fr) organizes a workshop in metabolomics under the auspices of the TRANSBIO SUDOE European Project. The workshop is dedicated to researchers, engineers or technicians with previous background in metabolomics, and wishing to strengthen their methodological expertise. The workshop includes 4 parallel practical courses:

a. Quantitative metabolomics by LC-MS/MS  
b. Metabolic fingerprinting by NMR  
c. Global metabolomics by high-resolution MS  
d. Lipidomics

Each practical course will take place from February 13th to 14th 2014 in the various locations of MetaToul. The number of participants per practical course is limited to 4, and the maximal number of participants is 16 (4 participants x 4 practical courses). A plenary session will be organized on the 13th evening with all participants.

Workshop program

Lipidomics:
Introduction to Lipidomic – preparation of a plasmatic extract – GC-FID/GC-MS/LC-MS injection – analytical processing of the analysis.

RMN:
Introduction to NMR-based metabolomics – preparation of 16 plasma samples – NMR spectroscopy of plasma samples – multivariate statistical analysis.

HRMS:
Introduction to MS-global metabolomic – LC-HRMS analysis – MS analysis of samples – multivariate statistical analysis.

Quantitative metabolomic:
Introduction to quantitative metabolomics – sampling and extraction – IC-MS/MS analysis – data processing and quantification of intracellular metabolites.

Plenary session (13th evening) & Dinner:
Welcome (Rémy Poupot, Transbio Coordinator)  
An introduction to MetaToul (JC Portais, MetaToul director)  
Reconstruction and analysis of metabolic networks (F. Jourdan, INRA Toulouse).  
Location: Maison SAMARAN, place Victor Hugo, Toulouse. A dinner (buffet) will be provide on site
Lipidomics Workshop

General introduction

Person in charge: Justine BERTRAND-MICHEL
Location: MetaToul-Lipidomics site (Rangueil Hospital)
Workshop presentation: Studying lipids in biological samples is quite complicated because of the very large diversity of lipid species. For this practical course, the following families can be investigated depending on specific requirements by the participants: fatty acids, phospholipids, sphingolipids, eicosanoids, sterols... The different steps of the analysis will be presented and performed by the participants:

a. Extraction
b. Pre-purification
c. Derivatization
d. Chromatographic analysis
e. Quantification

Program and schedules

**Day 1:**
13h : Lipidomic introduction (Justine BERTRAND-MICHEL) : lecture (1h30).
14h30 : Preparation of plasmatic-derivation extract (Justine BERTRAND-MICHEL) : practical course (2h).
16h30 : GC-FID/GC-MS/LC-MS injection (Pauline LE FAOUDER LC-Exactive : practical course 1h, Aude DUPUY LC-QQQ : practical course 1h, Justine BERTRAND-MICHEL GC/GC-MS : practical course 1h).

**Day 2:**
NMR Workshop

General introduction

**Person in charge:** Cécile CANLET  
**Location:** MetaToul-Axiom site (Saint Martin du Touch)

**Workshop presentation:** This practical course aims at the collection and analysis of metabolomic fingerprints of plasma samples (as a test sample) by NMR-based metabolomics. The different steps of the analysis will be presented and performed by the participants:

- a. Sample preparation
- b. 1H NMR spectroscopy of plasma samples
- c. Processing of NMR data
- d. Multivariate statistical Analysis of NMR data (Principal Component Analysis, Hierarchical Cluster Analysis, Partial Least Squares Regression)
- e. Identification of discriminating metabolites

Program and schedules

**Day 1:**

13h : Introduction to NMR-based metabolomics (Cécile Canlet) : *lecture (1h).*
14h : Preparation of 16 plasma samples (Cécile Canlet) : *practical course (1h30).*
15h30 : 1H NMR spectroscopy of plasma samples using the NMR autosampler Samplejet. Two NMR sequences will be recorded for each sample : noesypr1d and cpmgpr1d. 2D NMR experiments will be recorded for one sample to confirm the metabolite identification. (Cécile Canlet) : *practical course (1h30).*

**Day 2:**

9h : Processing of 1D NMR spectra : Fourier transformation, phase, calibration, baseline correction for each sample using TOPSPIN software. NMR spectra will be bucketed and integrated using AMIX software. Processing of 2D NMR spectra and identification of metabolites. (Cécile Canlet) : *practical course (2h30).*
11h30 : Introduction to Multivariate Statistical Analysis (Marie Tremblay-Franco) ? *lecture (45min).*
13h30 - 16h30 : Multivariate Statistical Analysis of NMR data obtained in the course using R software:
- Introduction to R software
- Principal Component Analysis (PCA)
- Hierarchical Cluster Analysis (HCA)
- Partial Least Squares Regression (PLSR)
- Identification of discriminating variables (loadings, VIP, anova, kruskal-Wallis test)
- Presentation of SIMCA-P software

Marie Tremblay-Franco et Cécile Canlet : *practical course (3h).*
## HRMS Workshop

### General introduction

Person in charge : Emilien JAMIN  
Location : site MetaToul-Axiom (Saint Martin du Touch)  
Workshop presentation : Together with NMR, mass spectrometry represents the most used technique for global metabolomic approaches. In this session, the high resolution mass spectrometry (HRMS) based global metabolomic approach will be illustrated, covering the following topics:

- Sample preparation
- Data generation using LC coupled to Orbitrap HRMS
- Filtering and pre-treatment of MS data (XCMS, Camera…)
- Multivariate statistical Analyses (Principal Component Analysis, Hierarchical Cluster Analysis, Partial Least Squares Regression)
- Metabolite identification

### Program and schedules

**Day 1 :**  
13h : Introduction to MS-based metabolomics (Laurent Debrauwer) : mass spectrometry general principles, MS analyzers : *lecture (1h).*  
14h : Sample preparation (Emilien Jamin) : *lecture (1h).*  
15h: Mass spectrometric analysis of samples on a hybrid high resolution mass spectrometer (LTQ-Orbitrap XL). The practical aspects concerning instrument preparation, sample injection sequence scheduling, data acquisition (MS, MS/MS) for global profiling and metabolite identification will be performed. (Emilien Jamin) : *practical course (2h).*

**Day 2 :**  
8h30 : Processing of MS data : XCMS software package. (Emilien Jamin, Jean-François Martin) : *practical course (2h).*  
10h30 : MS data cleaning (Emilien Jamin, Jean-François Martin) : *practical course (1h).*  
11h30 : Introduction to Multivariate Statistical Analysis (Marie Tremblay-Franco) : *lecture (0h45)* (common with the NMR working group).  
13h30 -16h30 : Multivariate Statistical Analysis of data obtained in the course using R software (common with the NMR working group) :  
  - Introduction to R software  
  - Principal Component Analysis (PCA)  
  - Hierarchical Cluster Analysis (HCA)  
  - Partial Least Squares Regression (PLSR)  
  - Identification of discriminating variables (loadings, VIP, anova, kruskal-Wallis test)  
  - Presentation of SIMCA-P software  
Marie Tremblay-Franco et Cécile Canlet : *practical course (3h).*
Quantitative metabolomic workshop

Person in charge : Lindsay PEYRIGA
Location : site *MetaToul-Réseaux Métaboliques* (INSA Rangueil)
Workshop presentation : This practical course aims at the absolute quantification of intracellular metabolites using LC-MS-MS methods. The different steps of the analysis will be presented and performed by the participants:
   a. Principles of quantitative metabolomics by Isotopic Dilution Mass Spectrometry
   b. Metabolite sampling: comparison of two methods (differential method and fast filtration)
   c. Sample preparation
   d. Ion Chromatography/MS-MS analysis
   e. Data processing and absolute quantification

Program and schedules

**Day 1 :**
13h : Introduction to Quantitative Metabolomics (Fabien Letisse) : *lecture (2h).*
15h: Sampling and extraction: quenching and extraction of intracellular metabolites belonging to *E.Coli* cells (Edern Cahoreau) : *practical course (2h30).*

**Day 2 :**
9h: Sample preparation for IC-MS/MS analysis + external calibration. Analysis of sample by IC-MS/MS. (Hanna Kulyk) : *practical course (2h).*
11h: Build of quantification method for raw data processing (Hanna Kulyk) : *practical course (1h).*
14h -16h30: Data processing and quantification of intracellular metabolites (Hanna Kulyk) : *practical course (2h30).*
   - Peak integration
   - Absolute quantification for each metabolites and each sample using IDMS approach