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97th Workshop on High-Resolution Respirometry & O2k-Fluorometry

2014 Sept 25-26 Lausanne, CH

Venue:
Ecole Polytechnique Federale de Lausanne (EPFL)

Host:
Sandi Carmen, Prof.
Laboratory of Behavioral Genetics
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Lecturer:
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The 97th O2k-Workshop on high-resolution respirometry and O2k-Fluorometry is an **Oxygraph-2k Workshop** held in cooperation with Prof. Carmen Sandi, Laboratory of Behavioral Genetics, Brain Mind Institute, Ecole Polytechnique Federale de Lausanne. The O2k-Workshop includes a basic introduction to instrumental setup, quality control of performance of the **OROBOROS Oxygraph-2k (O2k)** with integrated real-time data analysis, introducing new features of **DatLab 6**.

The workshop includes discussions on optimization of OXPHOS analysis in various mitochondrial (mt) preparations (permeabilized muscle fibres, tissue homogenate, isolated mitochondria). HRR provides information on cell respiration with simple phosphorylation control protocols in intact cells. State-of-the-art OXPHOS analysis is extended using mt-preparations, to evaluate coupling efficiencies and OXPHOS capacities with carbohydrate versus fatty acid substrates, and to diagnose defects in respiratory complexes of the electron transfer system and phosphorylation system. Novel developments are presented on **substrate-uncoupler-inhibitor titration (SUIT) protocols** in HRR using the **O2k-Fluorescence LED2-Module** for simultaneous measurement of hydrogen peroxide production (Amplex ultrared®). Discussions are extended on comparison of measurement of mt-membrane potential using Safranin (fluorometric) versus TPP^+ or $TPMP^+$ (potentiometric), and on perspectives of HRR in mitochondrial physiology.

Program IOC97

Thursday, September 25:

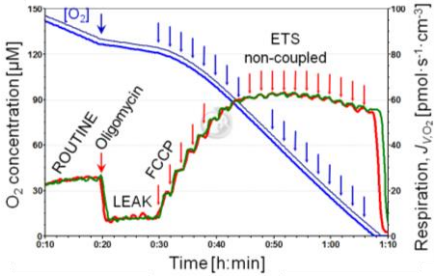
09:00 – 10:30 **A new Power-O2k Lab of Prof. Carmen Sandi: 4 O2k.**
- Instrumental setup, chamber assembly, OroboPOS – sensor service, quality control (group of Prof. Sandi).

10:30 *Coffee break – Registration of external participants*

11:00 – 11:15 **Carmen Sandi: Welcome to external participants**
Perspectives of mt-function – brain and mind.

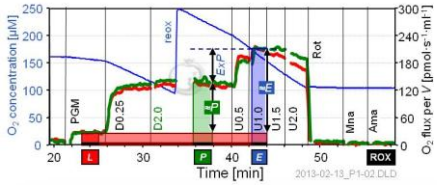
11:15 – 11:30 **Introduction of participants:** who is who?

11:30 – 12:30 **Erich Gnaiger:**
- Basic and new features of DatLab 6.
- Oxygen calibration.
- Oxygen flux and instrumental tests.



12:30 *Lunch*

13:30 – 15:30 **Experimental design.**
- Cell ergometry: from intact cells to mt-preparations.
- A challenge for simultaneous measurements of respiration and mt-membrane potential: solving a puzzle.



15:30 *Coffee Break*

16:00 – 17:30 **Titration-Injection microPump TIP2k:** From automatic instrumental background tests to steady-state control in respiratory experiments.

17:30 – 18:00 **Q&A session on HRR and OXPHOS analysis:** Design of experimental protocol - day 2.

Friday, September 26:

09:00 – 10:30 **Experiment:** HRR and O2k-Fluorometry – respiration and H_2O_2 production.

10:30 *Coffee break*

11:00 – 12:30 **Experiment** continued

12:30 *Lunch*

13:15 – 15:30 **DatLab analysis**

15:30 *Coffee break*

16:00 – 16:30 **Trouble shooting**

16:30 – 17:30 **Normalization of mt-respiration: flux control states and flux control steps.**

17:30 – 18:00 **Feedback – conclusions – stay connected** as a MiPNet Lab.

www.oroboros.at **www.bioblast.at** - the *information synthase* for Mitochondrial Physiology and high-resolution respirometry

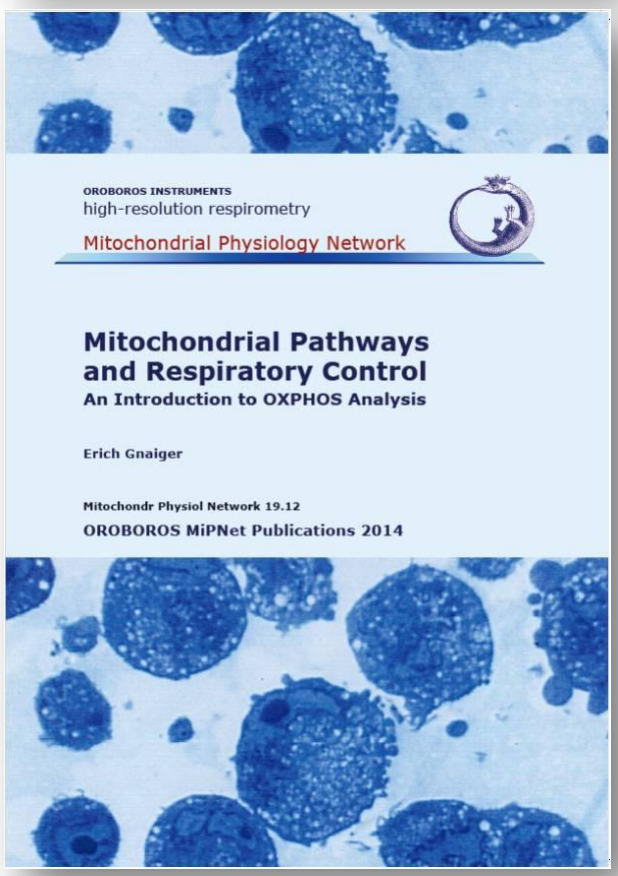
Recommended reading

O2k-Core Manual

New: **»O2k-Core Manual.pdf**

Mitochondrial pathways

Gnaiger E (2014) Mitochondrial pathways and respiratory control. An introduction to OXPHOS analysis. 4th ed. Mitochondr Physiol Network 19.12. OROBOROS MiPNet Publications, Innsbruck: 80 pp. »Open Access - ***handout to O2k-Workshop participants***



SUIT protocols for high-resolution respirometry

Pesta D, Gnaiger E (2012) High-resolution respirometry. OXPHOS protocols for human cells and permeabilized fibres from small biopisies of human muscle. Methods Mol Biol 810: 25-58. »Bioblast Access

Gnaiger E (2008) Polarographic oxygen sensors, the oxygraph and high-resolution respirometry to assess mitochondrial function. In: Mitochondrial Dysfunction in Drug-Induced Toxicity (Dykens JA, Will Y, eds) John Wiley: 327-352. »Bioblast Access

HRR and O2k-Fluorometry

» Manual: O2k-Fluorescence LED2-Module

Eigentler A, Fontana-Ayoub M, Gnaiger E (2013) O2k-Fluorometry: HRR and H_2O_2 production in mouse cardiac tissue homogenate. Mitochondr Physiol Network 18.05(01): 1-6.

» O2k-Fluorometry