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Workshop on high-resolution respirometry & O2k-Fluorometry

2016 March 06-07
Singapore, SG

Satellite workshop:
3rd International Symposium
New Frontiers in Cardiovascular Resarch.
March 08-09, 2016

Venue
National Heart Centre Singapore
5, Hospital Drive
Singapore, 169609

Host
O2k-Network Lab
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The 108th O2k-Workshop on high-resolution respirometry and O2k-Fluorometry is an **Oxygraph-2k Workshop** held in cooperation with our O2k-Network Lab in Singapore. This O2k-Workshop includes a basic introduction to quality control of instrumental performance of the **OROBOROS O2k** with integrated real-time analysis, introducing new features of **DatLab 6**.

The workshop includes a discussion on optimization of OXPHOS analysis in various mitochondrial (mt) preparations (permeabilized muscle fibres, tissue homogenate, isolated mitochondria). HRR provides information on respiration in intact cells with coupling control protocols. 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 electron transfer and phosphorylation systems. 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 Red®). Discussions are extended to measurements of mt-membrane potential using **O2k-MultiSensor Modules** and to perspectives of HRR in mitochondrial physiology.

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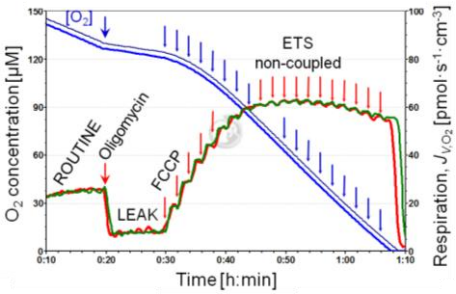
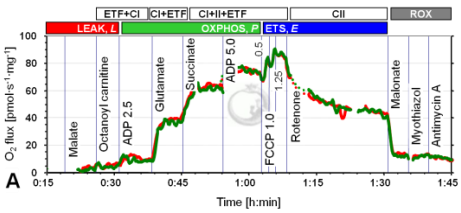
Program IOC

Sunday, March 06:

08:45	Registration
09:00 – 09:15	Welcome by Jean-Paul Kovalik
09:15 – 09:30	**Introduction of participants:** Who is who?
09:30 – 10:30	**Get started with the O2k.**
10:30	*Coffee break – Registration ctn.*
11:00 – 12:15	**Pros and cons of mt-preparations:** Coupling and substrate control of O_2 consumption and H_2O_2 production in homogenate, permeabilized fibres – or isolated mitochondria?
12:15 – 12:30	Permeabilized fibre preparation – what to take care of?
12:30	*Lunch*
13:15 – 14:00	**Coupling control protocol for intact cells.**
14:00 – 15:00	**Comprehensive OXPHOS analysis:** A challenge for simultaneous measurements of respiration and mt-membrane potential: solving a puzzle.
15:00 – 15:30	**Experimental setup 1:** OroboPOS - sensor quality control, calibration.
15:30	*Coffee Break*
16:00 – 17:00	**Experimental setup 2:** Calibration of O2k-Fluo Sensors
17:00 – 17:30	The Bioblast wiki and O2k-Network.
17:30 – 18:00	**Q&A session on HRR and OXPHOS analysis:** Design of experimental protocol - day 2.
18:30	*O2k-Workshop dinner at PappaSan, Dorsett Hotel*





Monday, March 07:

08:30 – 10:30	**Experiment:** HRR and O2k-Fluorometry – respiration and extracellular H_2O_2 production.
10:30	*Coffee break*
11:00 – 12:00	**Experiment** continued
12:00	*Lunch*
12:45 – 15:30	**Data analysis**
15:30	*Coffee break*
16:00 – 16:40	**Technical support & Open innovation**
16:40 – 18:00	**Feedback – conclusions – stay connected** as an O2k-Network Lab

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

Recommended reading

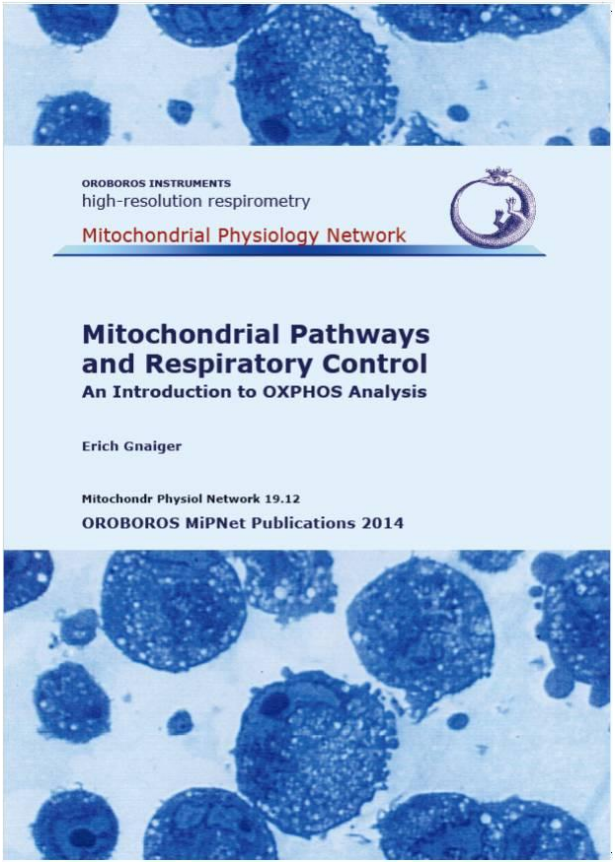
O2k-Core Manual:

»Bioblast link«

SUIT protocols for O2k high-resolution respirometry

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

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:327-52. »Bioblast link«



HRR and O2k-Fluorometry

»Manual: O2k-Fluo LED2-Module«

Makrecka-Kuka M, Krumschnabel G, Gnaiger E (2015) High-resolution respirometry for simultaneous measurement of oxygen and hydrogen peroxide fluxes in permeabilized cells, tissue homogenate and isolated mitochondria. Biomolecules 5:1319-38. »Bioblast link«

»O2k-Fluorometry Publications«

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. »Bioblast link«