OROBOROS O2k-Workshops

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110th Workshop on high-resolution respirometry & O2k-Fluorometry

2016 April 12-13
Melbourne, AU

Pre-conference workshop:
6th Exercise and Sport Science Australia Conference.
April 14-16, 2016

Venue:
Building P
Institute of Sport, Exercise and Active Living (ISEAL)
Victoria University
Ballarat Road
Melbourne, Australia

Host:
Nigel K. Stepto, PhD
David Bishop, Prof.
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http://wiki.oroboros.at/index.php/AU_Melbourne_Stepto_NK

Lecturers and tutors:
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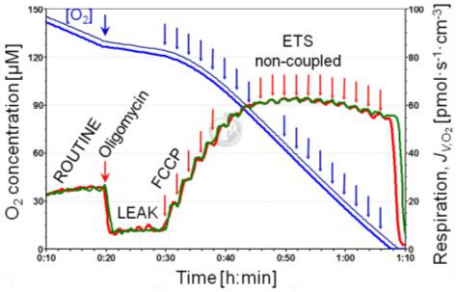
The 110th O2k-Workshop on high-resolution respirometry and O2k-Fluorometry is an **Oxygraph-2k Workshop** held in cooperation with one of our prominent O2k-Network Labs in Melbourne. This O2k-Workshop includes a basic introduction to quality control of instrumental performance of the **OROBOROS O2k** with integrated on-line analysis, introducing new features of **DatLab 7**.

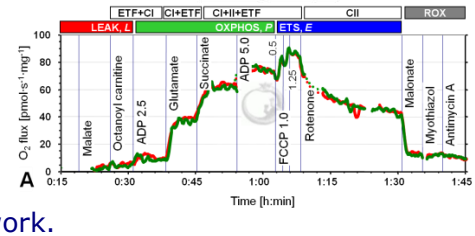
The workshop will include a discussion on optimization of OXPHOS analysis in various mitochondrial (mt) preparations (permeabilized muscle fibres, tissue homogenate, isolated mitochondria). HRR provides information on cell respiration with simple phosphorylation control protocols. State-of-the-art OXPHOS 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 red®). 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.

Programme

Tuesday, April 12:

08:45	Registration
09:00 – 09:15	Welcome by David Bishop
09:15 – 09:30	**Introduction of participants:** who is who?
09:30 – 10:00	**Get started with the O2k.**
10:00 – 10:30	**Human muscle biopsies preparation.**
10:30	*Coffee break – Registration ctn.*
11:00 – 12:15	**Pro's and con's 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 mt-preparations.**
14:00 – 15:00	**Comprehensive OXPHOS analysis: substrate-uncoupler-inhibitor titration (SUIT) protocols for coupling and substrate control.**
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*





Wednesday, April 13:

08:30 – 10:30	**Experiment:** HRR and O2k-Fluorometry with permeabilised fibres from rat – 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:

»Full text in Bioblast«

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. »Full text in Bioblast«

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. »Full text in Bioblast«

HRR and O2k-Fluorometry

»Manual: O2k-Fluo 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. »Full text in Bioblast«

»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. »Full text in Bioblast«