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113th Workshop on high-resolution respirometry & O2k-Fluorometry

2016 August 08-09
Havana, CU

Venue:
Centro de Investigación y Desarrollo de Medicamentos - CIDEM
Ave. 26, No. 1605 Boyeros y Puentes Grandes
CP 10600, La Habana, Cuba

Host:
Pardo Andreu Gilberto L, PhD
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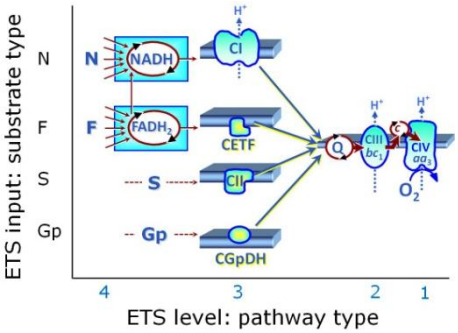


The **113th O2k-Workshop** on high-resolution respirometry and O2k-Fluorometry is held in cooperation with our first O2k-Network Lab in Cuba. This O2k-Workshop presents a basic introduction to the **OROBOROS Oxygraph-2k** with integrated real-time data analysis. We introduce the new software **DatLab 7** and the concept of a quality control system including the MitoFit interlaboratory proficiency test.

HRR provides information on cell respiration with basic coupling control protocols. State-of-the-art OXPHOS analysis is extended using mt-preparations (permeabilized muscle fibres, tissue homogenate, isolated mitochondria), to evaluate coupling efficiencies and OXPHOS capacities with electron transfer into the Q-junction converging from NADH, $FADH_2$, succinate and α-glycerophosphate (N,F,S,Gp), to diagnose defects in respiratory electron transfer system pathways and the 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

1 Monday, Aug 08

*printed in workshop materials

	Workshop Day 1	Weblink
08:30	*Registration*	
09:00-09:15	A **welcome** by Dr Andreu Gilberto Pardo and OROBOROS INSTRUMENTS	
09:15-09:30	**Introduction** of participants and their research interests	IOC113
09:30-10:30	**Get started with the O2k:** Overview with video clips.	O2k-Manual
10:30	*Coffee / Tea – Registration Continued*	
11:00-12:30	**Pro's and con's of mt-preparations**: Coupling and pathway control of O_2 consumption and H_2O_2 production in homogenate, permeabilized fibres – or isolated mitochondria?	
12:30	*Lunch*	
13:15-15:00	**Comprehensive OXPHOS analysis: substrate-uncoupler-inhibitor titration (SUIT) protocols for respiratory control by coupling and mitochondrial pathways, SUIT reference assay**	The Blue Book* SUIT reference protocol
15:00-15:30	**Experimental setup 1:** OroboPOS - sensor quality control, calibration.	
15:30	*Coffee / Tea*	
16:00-17:30	**Demo-Experiment:** High-resolution respirometry (and H_2O_2 production) with freeze dried baker's yeast.	Amplex Yeast HRR yeast RA*
17:30-18:00	**Q&A session on HRR and OXPHOS analysis:** Design of experimental protocol	
18:00-19:00	*Cultural Tour: „Havana City Tour"*	
19:30	*O2k-Workshop Dinner*	

2 Tuesday, Aug 09

	Workshop Day 2	Weblink
08:30-10:30	**Demo-Experiment:** high-resolution respirometry with homogenized tissue of rat brain	Burtscher (2015) Mitochondrion
10:30	*Coffee / Tea*	
11:00-12:00	**Experiment** continued	
12:00	*Lunch*	
12:45-15:30	**Data analysis & technical support**	
15:30	*Coffee / Tea*	
16:00-17:30	The Bioblast wiki and O2k-Network, Feedback & Conclusions	
17:30-19:30	*Farewell snack. Cultural activity at "Old Havana"*	

Recommended reading

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«

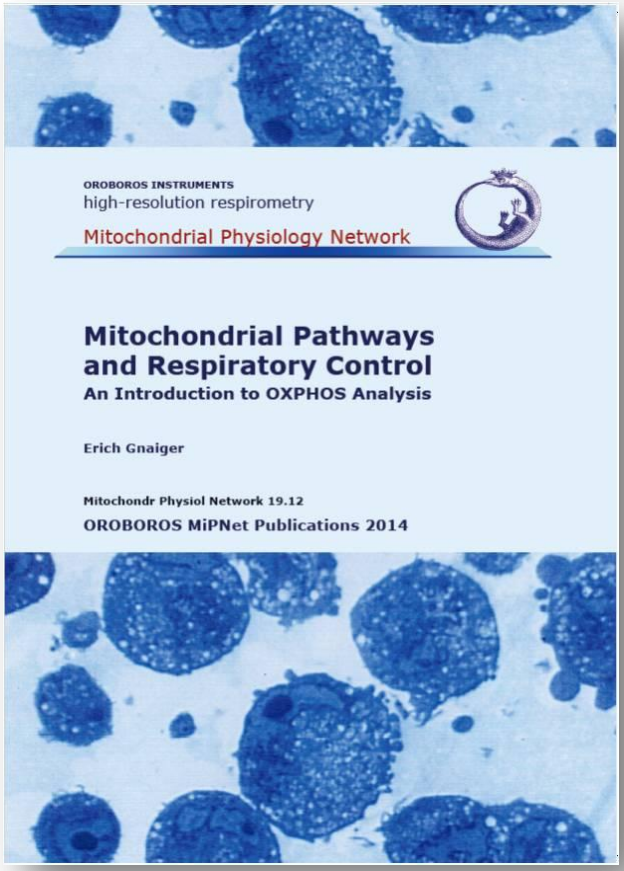
O2k-Core Manual:

»Full text in Bioblast«

SUIT protocols for O2k high-resolution respirometry

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«

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«



HRR with brain tisue

Burtscher J, Zangrandi L, Schwarzer C, Gnaiger E (2015) Differences in mitochondrial function in homogenated samples from healthy and epileptic specific brain tissues revealed by high-resolution respirometry. Mitochondrion 25:104-12. »Bioblast link«

COST Action CA15203 Mitochondrial fitness mapping
MITOEAGLE: Evolution - **A**ge - **G**ender - **L**ifestyle - **E**nvironment

Contribution to K-Regio project **MitoFit.**
The project MitoFit is funded by the Land Tirol within the program K-Regio of Standortagentur Tirol. www.mitofit.org

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