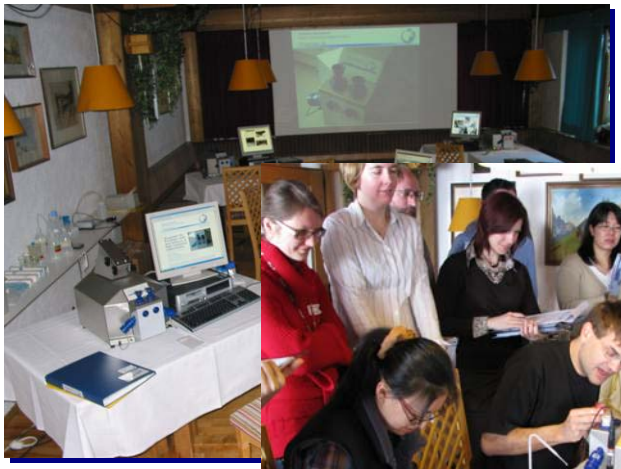
IOC54. *Mitochondrial Physiology Network* 14.15: 1-12 (2009)

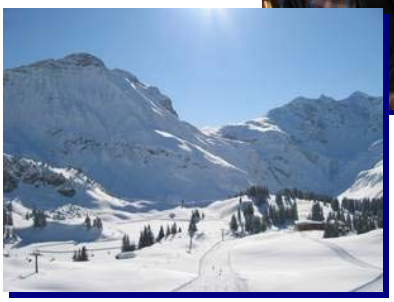
54th International Course on High-Resolution Respirometry

2009 Dec 11 – 16
Schröcken, Vorarlberg, Austria

The **54th O2k-Course** is the 21st presentation of high-resolution respirometry (HRR) in Schröcken since 1988. The O2k-Course includes experiments with biological samples, providing a practical overview of the **Oxygraph-2k**, with integrated on-line analysis by **DatLab 4.3** (new upgrade), applications of the **TIP-2k**, and perspectives of high-resolution respirometry in mitochondrial physiology. Emphasis is placed on hands-on applications by all participants.



An international team of experienced tutors guide small working groups step-by-step through the approach of HRR. Five Oxygraph-2k (10 chambers) are available for a do-it-yourself application of both hardware and software. Combined with an introduction and demo experiment, it is best to put the O2k into action yourself.



During lunch breaks, sufficient time is available for skiing or relaxing walks and talks, to enjoy the refreshing scenery of the secluded alpine environment, or use the spare time for specific tutorials. With DatLab 4.3 we accomplish data analysis on-line during the experiment, providing final results and their graphical presentation by the end of an experimental run. Thus we gain sufficient time to see the Titration-Injection microPump TIP-2k with new feedback-control in action and practice its simple and automatic operation.

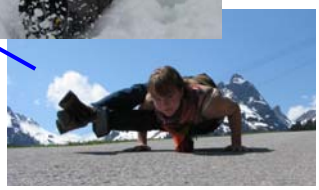
instruments@oroboros.at www.oroboros.at

Support

MITOFOOD COST Action Number FA0602 (Coordinator: Dr. Jaap Keijer, RIKILT-Institute of Food Safety, Wageningen University, The Netherlands.

Tutors

Erich Gnaiger, PhD (Innsbruck, AT)
Dan A Kane, PhD (Greenville, NC, US)
Clara De Palma, PhD (Milano, IT)
Dominik Pesta, MSc (Innsbruck, AT)
Constance Tweedie, MSc (Calgary, CA)
Dominique-Marie Votion, PhD (Liège, BE)



Programme IOC54

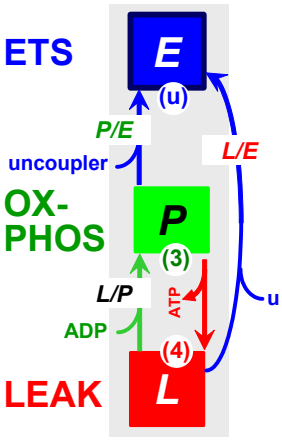
Day 1: Friday, December 11

15:00 Participants arriving in Bregenz: Meeting point at 4:00 pm in Bregenz train station; 1.1 hour drive to Schröcken and Hochtannberg Pass (Salober). Transfer by snowcat, check in at Hotel Körbersee.

18:30 Welcome reception Hotel Körbersee

19:00 Dinner

21:00 **Erich Gnaiger: Beyond respiratory states 3 and 4: Electron transport system (ETS), OXPHOS capacity and LEAK respiration - Experimental advances with high-resolution respirometry (HRR).**

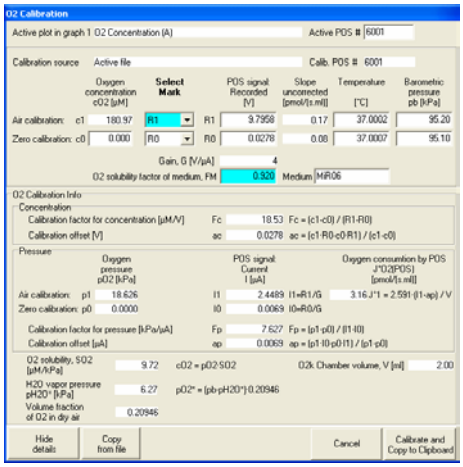


2.1.E 2.2.C

Day 2: Saturday, December 12

1.O2k

Principles of HRR - from switching on the Oxygraph-2k to the experimental result - with a little help from a friend: the O2k-Manual.



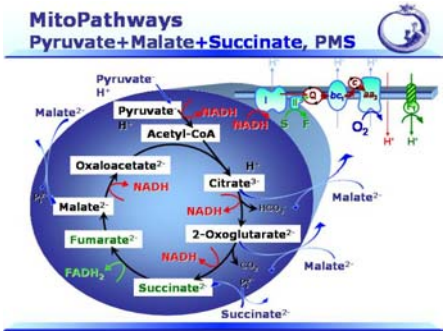
1.O2k.D

08:30 – 09:30 **The O2k system: Introduction and oxygen calibration of the polarographic oxygen sensors (OROBoPOS).**

09:30 – 10:30 **Hands-on: Oxygen sensor calibration with DatLab 4.3**

10:30 Coffee break

11:00 – 12:00 **Erich Gnaiger:**



Experimental protocols for substrate-uncoupler-inhibitor titrations (SUIT protocols): An introduction.

2.1.C

12:00 Lunch break - exercise

15:00 -16:30 Demo experiment – the Oxygraph-2k, a SUIT protocol and on-line DatLab analysis.

16:30 Coffee break

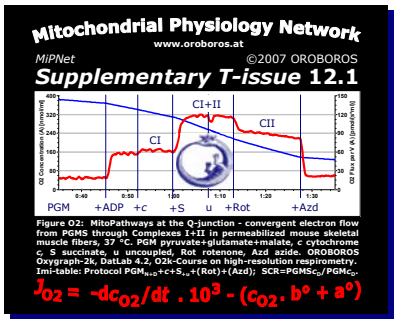
17:15 – 18:45 **Hands-on: Experiment with the Oxygraph-2k (five O2k - 10 chambers): SUIT protocol; on-line DatLab analysis.**

1.O2k.E

2.2.E

19:00 Dinner

21:00 **Discussion of results, protocol, DatLab analysis.**



Day 3: Sunday, December 13

08:30 – 09:00 **Erich Gnaiger: Instrumental background - introduction.**

09:00 – 12:00 **Hands-on (five groups): Oxygen calibration and instrumental background test with the Oxygraph-2k** - Washing and filling the O2k chambers with experimental media; air calibration; instrumental background *competition*, DatLab background analysis (see **2.4.C**. Instrumental background correction and accuracy of oxygen flux. *MiPNet* 14.6).

1.O2k.D

2.4.C

1.O2k.F

A. Instrumental background test for expeirments with cells and isolated mitochondria, from air saturation to zero oxygen concentration, wth automatic TIP-2k titration protocol.
B. Instrumental background test for experiments with permeabilized muscle fibers, in the high-oxygen range of 500 to 200 µM. Manual titration of hydrogen peroxide into MiR06 (MiR05 with catalase).

12:00 Lunch break - sports

16:00 Coffee/tea

2.2.A1/A2

16:30 – 17:15 **Background analysis – summary.**

17:15 – 17:45 **DatLab 4.3 – An overview.**

1.O2k.C

17:45 – 18:45 **Hands-on (five groups): Instrumental background analysis**

19:00 Dinner

Hot topics: MiPNet Session (10+10 min)
Chair: Constance Tweedie, Dan Kane (US)

21:00 - 21:20 **MiPNet 1: Marcelo O. Dietrich** (*Yale University School of Medicine, New Haven, US*) Sirtuin's action on the hypothalamic melanocortin system is enabled by UCP2.

21:20 - 21:40 **MiPNet 2: Etienne Hébert Chatelain** (*Université Victor Ségalen-Bordeaux 2, FR*) Src-mediated tyrosine phosphorylation; a new way of regulating mitochondrial bioenergetics?

21:40-22:00 **MiPNet 3: Sumbalová Zuzana** (*Comenius University, Bratislava, SK*) Current research in mitochondrial physiology.

Day 4 Monday, December 14

08:15 **Parallel group sessions - Introduction**

	Setup	POS Service	DatLab Analysis		
	O2k.A	O2k.B	O2k.C	O2k.D	O2k.E
08:30 – 09:15	Gr. 1	Gr. 2	Gr. 3	Gr. 4	Gr. 5
09:15 – 10:00	Gr. 5	Gr. 1	Gr. 2	Gr. 3	Gr. 4

10:00 Coffee

	Setup O2k.A	POS Service O2k.B	DatLab Analysis; general topics O2k.C	O2k.D	O2k.E
10:30 - 11:15	Gr. 4	Gr. 5	Gr. 1	Gr. 2	Gr. 3
11:15 – 12:00	Gr. 3	Gr. 4	Gr. 5	Gr. 1	Gr. 2
12:00 – 12:45	Gr. 2	Gr. 3	Gr. 4	Gr. 5	Gr. 1

13:00 Lunch - sports

16:00 Coffee/tea

16:30 - 17:30 **Working groups: Elaborate answers to the 'Questions for the O2k-Course'**

17:30 - 18:45 **Presentation of 'Answers for the O2k-Course' – Trouble shooting**

19:00 Dinner

Hot topics: MiPNet Session (10+10 min)
Chair: Clara De Palma (IT), Dominique-Marie Votion (BE)

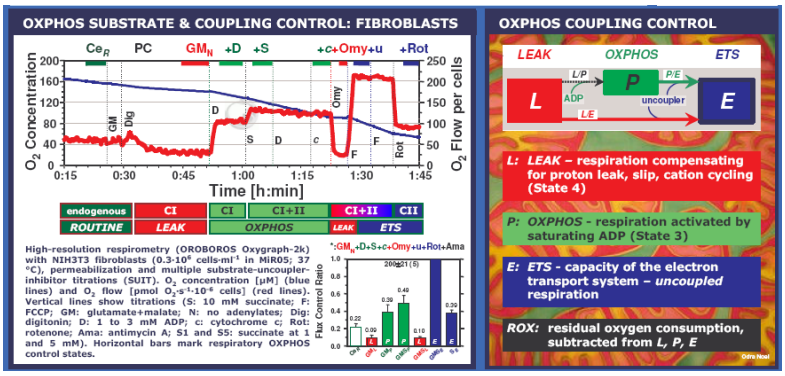
21:00 - 21:20 **MiPNet 4: Oleg Pak** (*University of Giessen, Germany*) Mitochondrial alterations in pulmonary arterial smooth muscle cells in pulmonary arterial hypertension.

21:20 - 21:40 **MiPNet 5: Nicolás Gutiérrez Cortés** (*Université Victor Ségalen-Bordeaux 2, FR*) Determination of the pathological effect of mitochondrial DNA mutations.

21:40-22:00 **MiPNet 6: Iyer Shilpa** (*Univ. of Virginia, Charlottsville, VA, US*) Current research in mitochondrial physiology.

Day 5: Tuesday, December 15

09:30 - 10:30



Erich Gnaiger: Experimental protocols.
a) Phosphorylation control protocol with intact cells: ROUTINE – LEAK – ETS
b) Diagnostic multiple SUIT protocols with mitochondrial preparations.

O2.2.A1
O2.2.E

10:30 Coffee break

11:00 - 12:00 **Erich Gnaiger: Principles and developments of the O2k-MultiSensorAnalyzer. NO, pH, and high-resolution TPP^+ electrode.**

12:00 Lunch break

14:00 - 15:00 **Dominique-Marie Votion: Respirometry with permeabilized fibres – horse skeletal muscle as an exercise and clinical model.**

15:00 - 16:00 **Special interest groups:**

A. Dominique-Marie Votion (BE) and Dominik Pesta (AT) Preparation of permeabilized fibres.

B. O2k and advanced DatLab features.

C. Practise DatLab 4 with demo data.
D. Operation of the Titration-Injection microPump – TIP-2k.

16:00 Coffee

16:30 – 17:00 ***Discussion - Summary – Conclusions***

17:15 Snowshoe walk (*rental of snowshoes*) to a welcome at the Alpmuseum: *Guided tour and special dinner cheese presentation by* ***Sommelier Maître Fromager Claudius Janner*** *(Immenstadt, Allgäu, DE) 20.- Euro.*

Alpmuseum uf m Tannberg, Batzen www.alpmuseum.at

Hotel Körbersee: Farewell party of IOC54

Day 6: Wednesday, December 16

Early morning: Departure

MiPNet Abstracts–

Hot topics in Mitochondrial Physiology

MiPNet 1. Sirtuin's action on the hypothalamic melanocortin system is enabled by UCP2.

Marcelo O. Dietrich, Catiele Antunes, Yongzhan Nie, Zhong-Wu Liu, Xiao-Bing Gao, Sabrina Diano, Diogo O. Souza, Qian Gao and Tamas L. Horvath
Program on Cell- and Neurobiology of Energy Metabolism, Section of Comparative Medicine, Yale University School of Medicine, New Haven, CT, USA, 06520.

Sirtuins are NAD^+-dependent class III deacetylases, highly conserved across species. SirT1, the mammalian ortholog of Sir2, has been implicated in the process that results in a prolonged lifespan as an effect of calorie restriction. The similarities between the action of sirtuins and calorie restriction raise the possibility that sirtuins may exert their effect, at least in part, by affecting brain circuits responsible for governing negative energy balance, the metabolic state promoted by calorie restriction. In the brain, the central melanocortin system is a master regulator of energy metabolism and its adaptive responses to a changing metabolic environment include synaptic and mitochondrial plasticity. Therefore, we attempted to examine whether SirT1 activity may play a role in these cellular adaptations in the hypothalamic melanocortin system.

Here we show that a central regulatory component in energy metabolism, the hypothalamic melanocortin system, is affected by sirtuins whereby they promote the activity and connectivity of this neuronal circuitry that is characteristic of negative energy balance. The suppression of SirT1 activity decreases the inhibitory tone on the anorexigenic POMC neurons, triggered either by negative energy balance or by the gut hormone ghrelin, leading to decreased food intake. This action of sirtuins requires an appropriate shift in the mitochondrial redox state, because in the absence of such an adaptation enabled by the mitochondrial protein, UCP2, sirtuin-induced cellular and behavioral responses are impaired. Because the melanocortin system is a key regulator of peripheral tissue function, the present data argues that sirtuins have a central mode of action on whole body physiology.

This project was supported by grants from CNPq, FINEP/MCT, INCT for Excitotoxicity and Neuroprotection (Brazil), ADA, JDRF, and NIH (USA).

1. Dietrich MO, Horvath TL (2009) The role of mitochondrial uncoupling proteins in lifespan. *Pflugers Arch*.
2. Dietrich MO, Andrews ZB, Horvath TL (2008) Exercise-induced synaptogenesis in the hippocampus is dependent on UCP2-regulated mitochondrial adaptation. *J. Neurosci.* 28: 10766-10771.
3. Andrews ZB, Liu ZW, Walllingford N, Erion DM, Borok E, Friedman JM, Tschöp MH, Shanabrough M, Cline G, Shulman GI, Coppola A, Gao XB, Horvath TL, Diano S. UCP2 mediates ghrelin's action on NPY/AgRP neurons by lowering free radicals (2008) *Nature* 454: 846-851.

MiPNet 2. Src-mediated tyrosine phosphorylation; a new way of regulating mitochondrial bioenergetics?

Etienne Hébert Chatelain, Thierry Letellier and Jeanne Dachary-Prigent
INSERM-U688 Physiopathologie Mitochondriale, Université Victor Ségalen-Bordeaux 2, 146 rue Léo Saignat, F-33076 Bordeaux-Cedex, France.

Mitochondria produce the most part of the energy consumed by cells through oxidative phosphorylation. Since energetic demands of cells depend on their functions, energy production must be tightly regulated. Phosphorylation (or dephosphorylation) is one of the major regulation pathways of enzymes. Several phosphorylated proteins are found in all mitochondrial compartments and numerous kinases and phosphatases are specifically targeted to this organelle. Recent work had shown that cyclic AMP, which is produced in mitochondria, is able to phosphorylate some OXPHOS components, regulating ATP production [1]. For example, it was previously shown that cAMP-dependent phosphorylation of ESSS subunit of Complex I increase its enzymatic activity and the overall mitochondrial respiration [1]. Inversely, cAMP-dependent phosphorylation of tyrosine 304 in subunit I of Complex IV inhibits its activity. Phosphorylation of cytochrome *c* on tyrosine 95 also decreased the activity this complex [1]. Phosphorylation on tyrosine residue now appears important among phosphorylation pathways in mitochondria, especially since the discovery, in rat brain mitochondria, of tyrosine kinases of the Src family, which are the most active kinases in those mitochondria [2], and tyrosine phosphatases, such as SHP2 and PTP1B which regulate the activation of Src kinases [3]. Src-mediated tyrosine phosphorylation is emerging as an important pathway for the regulation of mitochondrial bioenergetics.

The first part aimed at understanding the impact of Src and PTP1B, by using inhibitors of those enzymes, on mitochondrial respiration (oxygraphy) and on enzymatic activities of OXPHOS components (spectrophotometry). Mitochondrial Src was upregulated by PTP1B (as in the cytosol). In addition, inhibition of Src decreased mitochondrial respiration. Finally, catalytic capacities of OXPHOS components appeared to be partly dependent of Src-mediated tyrosine phosphorylation.

In order to understand the precise action mode of this kinase, the second part of our project will aim to identify the specific Src targets among OXPHOS components. These residues will be identified by mass spectrometry of OXPHOS complexes immunoprecipitated after exposition with purified Src kinase. Interaction between Src and its subtrates will be visualized by electronic microscopy.

The third objective will be aimed at understanding the importance of this Src-related pathway in mitochondrial pathologies. A first cellular model will be exposed to different inhibitors of OXPHOS components and of Src kinase. In order to understand the cellular response over these deficits, each step of mitochondrial biogenesis and physiology will be examined; (i) expression of mitochondrial genes (real time PCR); (ii) subunits formation and assembly of OXPHOS complexes (BN/SDS-PAGE and western blot); (iii) catalytic capacities of OXPHOS complexes (spectrophotometry); (iv) global mitochondrial respiration (high-resolution respirometry) and, finally, (v) Src-related phosphorylation status of different OXPHOX components. This thesis, by studying phosphorylation of OXPHOS components, is essential to a better understanding of mitochondrial physiology. Moreover, studying the consequences of those phosphorylations is also essential to increase our understanding of mitochondrial pathologies.

1. Huttemann M et al (2007) Regulation of mitochondrial oxidative phosphorylation through cell signaling. *Bioch. Biophys. Res. Comm.* 331: 1-14.
2. Tibaldi E et al (2008) Src-tyrosine kinases are major agents in mitochondrial tyrosine phosphorylation. *J. Cell. Biochem.* 104: 840-849.
3. Roskoski R (2004) Src protein-tyrosine kinase structure and regulation. *Bioch. Biophys. Res. Comm.* 324: 1154-11164.

MiPNet 4. Mitochondrial alterations in pulmonary arterial smooth muscle cells in pulmonary arterial hypertension.

Oleg Pak, Natascha Sommer, Philipp Krug, Sharon Waisbrod, , Hossein A. Ghofrani, Ralph T. Schermuly, Werner Seeger, Friedrich Grimminger, Norbert Weissmann
University of Giessen, Lung Center, Medical Clinic II, Justus-Liebig-University, Gießen, Germany

Vascular remodelling with proliferation of pulmonary arterial smooth muscle cells (PASMC) leads to pulmonary arterial hypertension (PAH), a rapidly progressive disease

with bad prognosis which ultimately results in right heart failure. Mitochondria have been suggested to play a crucial role in development of PAH due to their central function in the newly developed concept of cancer like proliferation of PASMC in PAH. We therefore investigated alterations in the mitochondrial respiratory system in PASMC in the model of monocrotaline and hypoxia induced PAH.

Mitochondrial function was measured respirometrically after exposure of PASMCs to hypoxia (1% O_2 and 7% O_2 for 24 h and 48 h) and in PASMCs isolated from rats four weeks after intraperitoneal administration of monocrotaline. Mitochondrial membrane potential was measured by fluorescence microscopy. Gene regulation possibly underlying the identified alterations were investigated by polymerase chain reaction (PCR).

In the monocrotaline model, as well as after exposure to hypoxia a decrease in mitochondrial respiration could be detected. Mitochondrial membrane potential was increased. In both models an increase of pyruvate dehydrogenase 1 and the PASMC specific cytochrome *c* oxidase subunit IV isoform 2 was detected by PCR. After reexposure of the PASMC to normoxia the functional alterations of mitochondria in the hypoxia model were reversible.

Mitochondrial phenotype is similarly altered in the monocrotaline and hypoxia model of PAH. This result stresses the crucial role of mitochondria in development of PAH and offers the possiblity for new treatment strategy in PAH.

MiPNet 5. Determination of the pathological effect of mitochondrial DNA mutations.

Nicolás Gutiérrez Cortés[1], Claire Pertuiset[1], Denis Pierron[1], Thierry Letellier[1], Delphine Feldmann[2], Christophe Rocher[1]

[1] *U688 - INSERM, Physiopathologie Mitochondriale, Université Victor Segalen Bordeaux 2, Bordeaux, France.*

[2] *Laboratoire de Biochimie et Biologie Moleculaire, Hôpital A. Trousseau, Paris, France.*

It has been shown that certain isolated deafness of maternal inheritance can be caused by mtDNA mutations. In collaboration with Delphine Feldmann's laboratory at the Trousseau Hospital in Paris, specialized on the study of deafness, we characterized some mtDNA mutations found in patients of this special type of deafness, in order to determine if they can be the cause of the pathology. These mutations are: C3388A, located in the coding sequence of subunit 1 of Complex I; G8078A, located in the coding sequence of subunit 2 of Complex IV; G12236A, affecting the transfer RNA serine; and G15077A, located in the coding sequence of cytochrome *b* of Complex III.

These mutations were found by a total sequencing of the mitochondrial genome, using a microarray technique developed by Affymetrix, Mitochip. To determine if they are responsible for the pathology, we constructed cybrids (CYtoplasme hybrid). This technique consists of a cellular fusion between a cell containing mitochondrial DNA, and another cell with no mtDNA but with a known genomic DNA, which allows us to place patient's mtDNA into a known nuclear background. In this way, mitochondrial dysfunction could only be caused by patient's mtDNA. These cybrids allowed us to carry out enzymological studies.

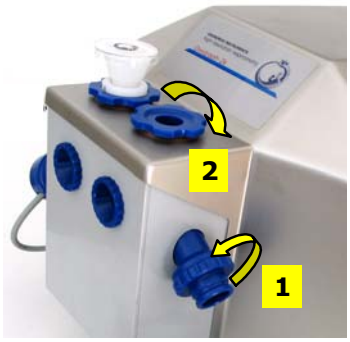
We showed that some of these mutations caused respiratory chain dysfunctions. Specially mutation C3388A, which causes a clear dysfunction of Complex I, and mutation G8078A, causing Complex III and Complex IV dysfunctions.

Our results suggest that these mutations cause a cellular metabolism deficit, which implies that they could be the origin of the pathology. These studies will be completed by protein analysis, in order to study the consequences of these mutations on respiratory Complex assembly, as well as polarographic analysis, in order to study the global activity of the respiratory system.

1. Chomyn A (1996) Platelet-mediated transformation of human mitochondrial DNA-less cells. *Methods Enzymol.* 264: 334-339.
2. Leveque M, Marlin S et al (2007) Whole mitochondrial genome screening in maternally inherited non-syndromic hearing impairment using a microarray resequencing mitochondrial DNA chip. *Eur. J. Hum. Genet.* 15: 1145-1155.
3. Rocher C, Taanman JW et al (2008) Influence of mitochondrial DNA level on cellular energy metabolism: Implications for mitochondrial diseases. *J. Bioenerg. Biomembr.* 40: 59-67.

Questions for the O2k-Course

The O2k-Manual provides answers to many of these questions ([⊘] Chapter numbers in the O2k-Compendium on the CD) – and you find more information on www.oroboros.at …



1. Oxygraph-2k assembly [⊘1.O2k.A]

1.1. What is the most important consideration for positioning the glass chamber during assembly of the O2k?
1.2. How do you detect an oxygen leak in the chamber?

2. Polarographic oxygen sensor (POS)

2.1. Why is it important to check the non-calibrated raw signal (voltage, after current-to-voltage conversion) of the polarographic oxygen sensor, and how can you quickly see the raw signal on-line?
2.2. The sensor voltage is above 9.9 V. What should you do?
2.3. Why is it important to maintain an extremely constant temperature in and around the O2k-chamber?
2.4. Does the POS respond to oxygen concentration, c_{O2} [µmol·dm^{-3} = µM], or partial oxygen pressure p_{O2} [kPa]?

3. POS calibration [⊘1.O2k.D]

3.1. How many calibration points are required for proper calibration of the polarographic oxygen sensor (POS)?
3.2. Should the chamber be open or closed during POS calibration?
3.3. What is an acceptable voltage (raw signal) of the POS at (a) air calibration, and (b) zero oxygen calibration, and how are these raw signals affected by the gain setting?
3.4. Why should you check the raw voltage during calibration?
3.5. How do you perform a zero oxygen calibration?
3.6. The oxygen solubility, S_{O2} [µM·kPa^{-1}], relates oxygen concentration to partial pressure. How is S_{O2} related to the solubility factor, F_M? Which variables need to be considered for estimation of the oxygen solubility of an aqeous solution, for example of mitochondrial respiration medium MiR06? [⊘2.4.A]
3.7. When is the oxygen calibration of a POS preferentially performed?
3.8. How long does it take approximately (5, 15, 30 or 45 min) to perform an oxygen calibration at air saturation, after the O2k is switched on (at experimental temperature in the range of 20 to 37 °C)?
3.9. Do you have to consider the instrumental background when performing an oxygen calibration of the POS at zero oxygen concentration?
3.10. Do you need to consider the instrumental background when performing an oxygen calibration of the POS at air saturation?
3.11. Does the oxygen signal have to be stable for an oxygen calibration of the POS?
3.12. How do you define POS signal stability? [⊘1.1.D]
3.13. Do you have to perform a zero oxygen calibration of the POS before air calibration?
3.14. Can you calibrate the POS with biological sample and respiratory activity in the aqueous solution, when equilibration is performed with a gas phase in the chamber and stability of the signal is observed?
3.15. What is the difference between static calibration [⊘1.O2k.D] and dynamic sensor calibration (time constant – for advanced users)? How can you use a dynamic calibration (stirrer test) as a quick sensor test? [⊘1.O2k.G]

4. POS Service [⊘1.O2k.B]

4.1. What should be done if the sensor connector threads appear dark and dirty?
4.2. The POS membrane box appears to have two types of membranes, which one should be applied to the sensor?

4.3. How can you avoid creating bubbles when filling the electrolyte reservoir of the POS?
4.4. Can the ammonia treatment be applied repeatedly?
4.5. How can you check sensor performance?
4.6. What precautions should be taken when handling the sensor connector?

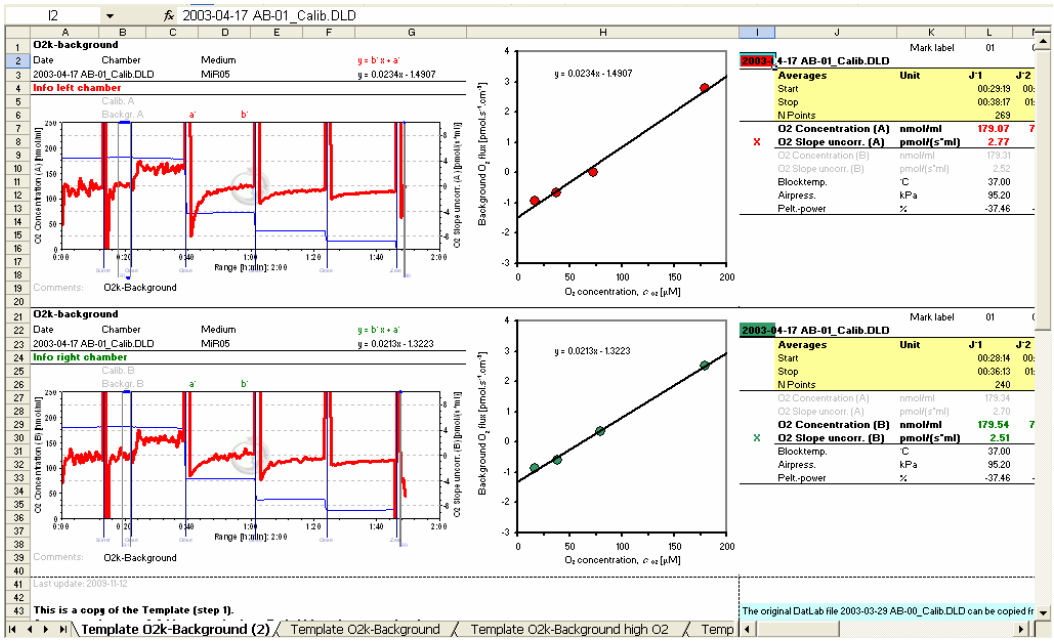
5. Cleaning of the Chamber [2.4.A]

5.1. Which solution should be placed in the chamber when the O2k is not in use (i.e. overnight, for a few days)?
5.2. Can detergents be used to clean the chamber and the PVDF stoppers?
5.3. What is the recommended cleaning procedure between experimental runs?
5.4. The glass chambers appear to have surface residue. Can this be removed, what is the procedure?
5.5. The stirring bar gets stuck. What can be done?

6. Instrumental background test [1.O2k.E; 2.4.C]

6.1. Does the oxygen signal have to be stable for setting a mark in an instrumental background test?
6.2. Does the oxygen flux have to be constant for setting a mark in an instrumental background test?
6.3. How do you define flux stability? Is a flat horizontal red line always an indication of a stable flux?
6.4. Do you need to determine instrumental background flux at air saturation and zero oxygen concentration?
6.5. Do you need to calibrate the POS before performing an instrumental background calibration?
6.6. We use the symbol *a*° for the intercept at zero oxygen concentration, and the symbol *b*° for the slope of background oxygen flux as a function of oxygen concentration. In the analysis of instrumental background, we have obtained 0.022 and -1.7. Which value is *a*° and *b*°, respectively?
6.7. Does the background-corrected flux have to be zero when the oxygen signal is stable?
6.8. How often do you have to check the instrumental background?

This was the wrong protocol for shoes as a background of snow-shoes at the walk to the Alpmuseum.



Literature

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. – *A methodological introduction into high-resolution respirometry, with focus on*

- Polarographic oxygen sensor and traditional oxygraphy
- High-resolution respirometry: The Oxygraph-2k
- Calibration of Polarographic Oxygen Sensors and Oxygen Concentration in Respiration Media at Air Saturation
- From Oxygraph Slopes to Respiratory Flux Corrected for Background Effects
- Phosphorylation control protocol with intact cells
- Titration Steps of the PC Protocol
- Experimental Example for the PC Protocol
- Flux Control Ratios from the PC Protocol
- Intact cells, permeabilized cells and tissue, or isolated mitochondria?

Gnaiger E (2009) Capacity of oxidative phosphorylation in human skeletal muscle. New perspectives of mitochondrial physiology. *Int. J. Biochem. Cell Biol.* 41: 1837–1845.

- Respirometry with permeabilized fibres and isolated mitochondria
- Convergent CI+II electron input and *OXPHOS* capacity
- Tissue-*OXPHOS* capacity in human permeabilized muscle fibres and isolated mitochondria
- Tissue-OXPHOS capacity and functional diversity

Gnaiger E (2001) Bioenergetics at low oxygen: dependence of respiration and phosphorylation on oxygen and adenosine diphosphate supply. *Respir. Physiol.* 128: 277-297. – *A detailed introduction into high-resolution respirrometry with particular emphasis on kinetics and measurements at low oxygen:*

- Mitochondrial kinetics measured by high-resolution respirometry
- Calibrations and corrections for response time and instrumental background
- Steady-state injection respirometry
- Mitochondrial respiratory control at low oxygen
- Apparent oxygen affinity and catalytic efficiency of mitochondrial respiration
- Effect of ADP and oxygen limitation on ADP/O2 flux ratios
- The low-oxygen environment of the cell: Mitochondria between hypoxic and oxidative stress

Gnaiger E, Kuznetsov AV, Schneeberger S, Seiler R, Brandacher G, Steurer W, Margreiter R (2000) Mitochondria in the cold. In: *Life in the Cold* (Heldmaier G, Klingenspor M, eds) Springer, Heidelberg, Berlin, New York: 431-442. – *Isolated mitochondria and permeabilized muscle fibers, MiR05.*

- Optimization of mitochondrial cold storage
- Mitochondrial respiration medium, MiR05
- Mitochondrial cold ischemia-reperfusion injury

Renner K, Amberger A, Konwalinka G, Kofler R, Gnaiger E (2003) Changes of mitochondrial respiration, mitochondrial content and cell size after induction of apoptosis in leukemia cells. *Biochim. Biophys. Acta* 1642: 115-123. – *Intact cells, cytochrome c oxidase, cytochrome c test, respiration per million cells, per citrate synthase, per mg protein, or per cytochrome c oxidase activity.*

Further information: Introductory course material is available on our homepage www.oroboros.at, with the following sections:

1. **Oxygraph-2k and Manual**
2. **MiPNet Protocols** - www.oroboros.at/index.php?o2k-protocols
3. **O2k-Publications**
4. **Mitochondrial Physiology Network**

Accomodation and Location

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