IOC55. *Mitochondrial Physiology Network* 15.1: 1-9 (2010)

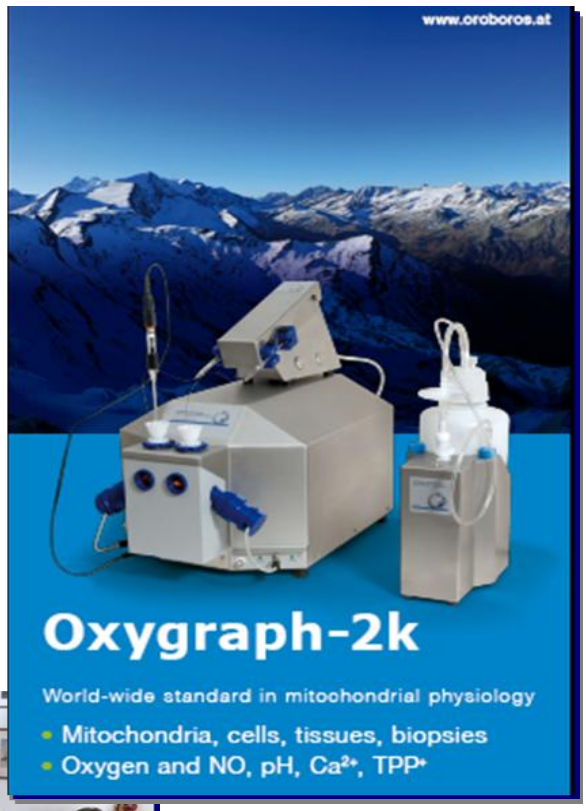
Last update: 2010-03-30

55th Workshop on High-Resolution Respirometry

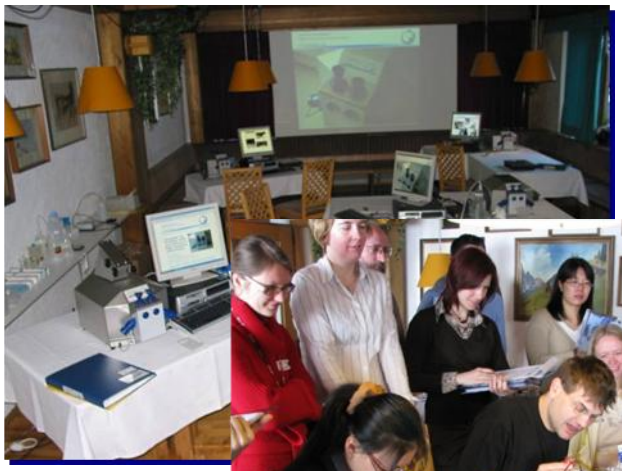
International Oxygraph-2k Workshop

2010 April 7 – 12

Schröcken, Vorarlberg, Austria



The **55th** Workshop on High-Resolution Respirometry (HRR) is the 22nd international oxygraph course held in Schröcken since 1988. The workshop 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** with new feedback-control, 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 guides 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 hardware and software. It is best to put the O2k into action yourself. With DatLab 4.3 we accomplish data analysis on-line, with final results and graphical presentations by the end of an experimental run.

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.

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, AT
Dominik Pesta, AT
Kathrin Renner-Sattler, AT/DE
Suzana Sumbalová, SK/AT
Anita Wiethüchter, AT (*admin.*)
Esther Phielix, NL/DE *(guest tutor)*

Programme IOC55

Day 1: Wednesday, April 07

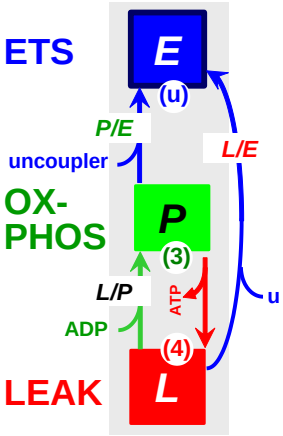
15:00 Arrival in Bregenz: Meeting point Bregenz train station at 3:00 pm; 1.1 hour bus drive to Schröcken and Hochtannberg (Salober). Transfer to Hotel Körbersee.

18:30 Welcome reception at Hotel Körbersee

19:00 Dinner

21:00-21:20 **Erich Gnaiger: IOC55 – a celebration** (see page 9).

21:20-22:00 **Introductions of participants and their research interests.**



Day 2: Thursday, April 08

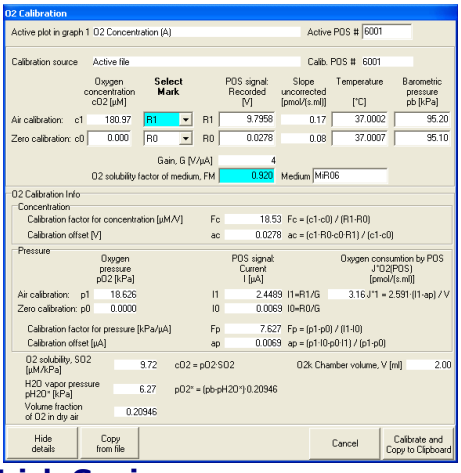
1.O2k.A **Principles of HRR - with a little help from a friend: the O2k-Manual.**

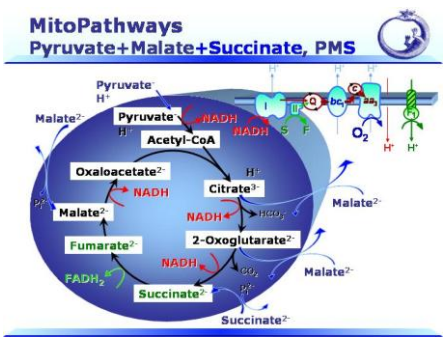
08:30 – 09:30 **The O2k and HRR: Introduction and oxygen calibration of the polarographic oxygen sensors (OROBoPOS).**

1.O2k.D

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

14:30 -16:15 **Demo experiment – the Oxygraph-2k and on-line DatLab analysis.** Yeast as a HRR-model.

16:15 Coffee break

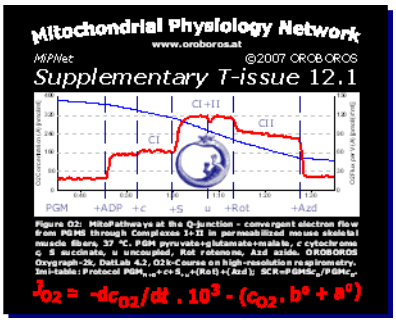
16:45 – 18:45 **Hands-on: Experiment with the Oxygraph-2k (five O2k - 10 chambers) and on-line DatLab analysis.**

1.O2k.E

2.2.E

19:00 Dinner

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



Day 3: Friday, April 09

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

09:30 – 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 experiments with cells and isolated mitochondria, from air saturation to zero oxygen concentration, with automatic TIP-2k titration protocol.

B. Instrumental background test for experiments with permeabilized muscle fibres, in the high-oxygen range of 400 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

1.O2k.C

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

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

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

19:00 Dinner

Hot topics: MiPNet Session (10+10 min)
Chair: Esther Phielix, Kathrin Renner-Sattler

21:00 - 21:20 **MiPNet 55.1: Van Bergen Nicole** (*Australia*) Decreased mitochondrial oxidative phosphorylation in autosomal dominant optic atrophy.

21:20 - 21:40 **MiPNet 55.2: Gonçalves Renata de Lima Sales** (*Brazil*) Mitochondrial uncoupling in the mosquito *Anopheles gambiae* enhances *Plasmodium* infection.

Day 4 Saturday, April 10

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

	Setup O2k.A	POS Service O2k.B	DatLab Analysis O2k.C	DatLab Analysis O2k.D	Fibre prep. 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 O2k.C	O2k.D	Fibre prep. 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

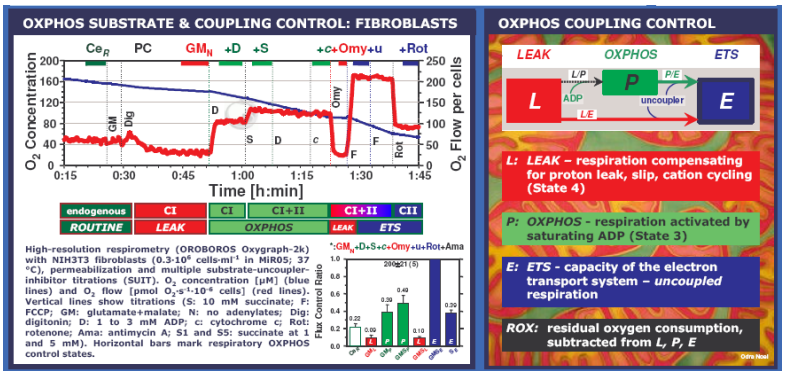
16:30 - 17:30 **Working groups: Elaborate answers to the 'Questions for the O2k-Course'**

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

19:00 Dinner

Day 5: Sunday, April 11

08:30 - 10:30



Erich Gnaiger: Experimental protocols.

a) Phosphorylation control protocol with intact cells: ROUTINE – LEAK – ETS
b) Diagnostic SUIT protocols with mt preparations.

O2.2.A1
O2.2.E

10:30 Coffee break

11:00 - 12:00 **Kathrin Renner-Sattler: Trouble shooting in HRR**

12:00 Lunch break

14:00 - 15:00 **Dominik Pesta: Permeabilized muscle fibres – preparation and HRR. MiPNet 3.**

15:00 - 16:00 **Open topics:**

- **A. O2k MultiSensor overview.**
- **B. O2k and advanced DatLab 4.3 features.**
- **C. Practise DatLab 4.3 with demo data.**
- **D. Operation of the Titration-Injection microPump – TIP-2k.**

16:00 **Snowshoe walk** (*rental of snowshoes*) **to the Alpmuseum**: *Guided tour and reception: € 15*

Alpmuseum uf m Tannberg, Batzen www.alpmuseum.at

19:00 Dinner

21:00 ***IOC55 feedback - discussion - summary – conclusions***
Farewell party of IOC55

Day 6: Monday, April 12

Early morning: Departure

MiPNet Abstracts–

Hot topics in Mitochondrial Physiology

MiPNet 55.1. Decreased mitochondrial oxidative phosphorylation in autosomal dominant optic atrophy

NJ Van Bergen, IA Trounce, DA Mackey, AW Hewitt, G Kong, JG Crowston
Centre for Eye Research Australia, University of Melbourne, Melbourne, Australia

Purpose: Large variation in the degree of vision loss is observed among Autosomal Dominant Optic Atrophy (ADOA) siblings who harbour identical mutations in OPA1. The cause for this variation in phenotype is not known. We performed detailed analysis of mitochondrial oxidative phosphorylation in ADOA patients with good vision compared to patients with significant visual loss.

Methods: EBV transformed lymphoblasts of ADOA patients with poor (<6/36, n=10) or good (>6/9, n=10) visual acuities or non mutation carrier controls (n=20) were established. Mitochondrial OXPHOS enzyme activity was measured spectrophotometrically from sonicated mitochondria. To determine mtDNA content southern blots were probed with primers overlapping the entire human mtDNA genome. Changes in protein expression level of OPA1, nuclear and mitochondrial subunits of Complex IV and actin were measured by western blot. Statistical analysis was measured by single factor ANOVA.

Results: Measuring OXPHOS in all ADOA patients compared to controls we found a significant decrease in complex I activity (P=0.05) and significant increase in II+III linked activity (P<0.01) whilst no changes in activity of complexes II, III or IV. Southern blotting of mtDNA content revealed no significant difference in mtDNA copy number across patient samples. Total OPA1 content detected by western blot was significantly decreased by 74% in both poor (P<0.01) and good (P<0.01) vision ADOA compared to controls, but no difference between good and poor vision. Interestingly in good vision ADOA compared to poor vision ADOA there was a significant increase in the II+III linked (P=0.05) and Complex IV activity (P=0.026). Furthermore there was upregulation of both nuclear (P=0.063) and mitochondrial encoded (P=0.025) Complex IV subunits in patients with good vision compared to poor vision.

Conclusion: We describe impaired Complex I activity in lymphoblast mitochondria of ADOA patients, and in good vision ADOA show an increase in activity of Complexes II+III as well as IV, which correlated with the increased protein levels of Complex IV. Identifying specific defects in mitochondrial function that are associated with vision loss in ADOA may provide a method for predicting which individuals are most at risk of losing vision.

MiPNet 55.2. Mitochondrial uncoupling in the mosquito *Anopheles gambiae* enhances *Plasmodium* infection

RLS Goncalves, JHM Oliveira, MF Oliveira, PL Oliveira, C Barillas-Mury
Institute of Biomedical Medicine, Federal University of Rio de Janeiro, Brazil

Malaria is a deadly disease that affects millions of people worldwide and is transmitted by anopheline mosquitoes. The mosquito immune system is capable to mount an efficient anti-plasmodium response that involves the production of toxic reactive species (ROS) in the midgut. Mitochondria are an important source of ROS and its contribution to ROS-based immunity has been overlooked. Here, we performed the first characterization of *Anophles gambiae* respiration in the midgut and identified a mitochondrial transporter, AgMC1, that is up-regulated upon *Plasmodium berghei* infected blood meal. The silencing of AgMC1 resulted in mitochondrial uncoupling, decreased ROS generation and enhanced

plasmodium susceptibility. This work set the basis of a novel concept linking the mitochondrial metabolism and mosquito immune response, pointing the regulation of mitochondrial ROS as an effective immune tool for *A. gambiae* against *Plasmodium* infection.

MiPNet 55.3. Investigation of muscle metabolism of the *M. quadriceps* via 31P MRS and high-resolution respirometry in connection with exercise training in normoxia and hypoxia: methodological aspects of muscle fibre preparation for high-resolution respirometry

D Pesta, M Faulhaber, M Burtscher, M Schocke, E Gnaiger

Skeletal muscle is a highly adaptable tissue that can adjust to different external stimuli. In the present study the impact is being studied of altered environmental conditions (normoxia and hypoxia) as well as training regimes (strength and endurance training) on parameters of muscle metabolism such as mitochondrial capacity.

For assessing these parameters, 60 healthy and not specifically trained subjects are taking part in a specific strength and endurance training program lasting for 12 weeks. The collective is split into a normoxic and a normobaric intermittent hypoxic (FiO2=0.12) training group. At baseline, subjects will perform an in-vivo phosphorus-31 magnetic resonance spectroscopy (31P MRS) of the quadriceps muscles during dynamic leg-extension exercise. Subsequently, endurance and strength capacities of the subjects will be determined via motor performance tests. Biopsy samples from the vastus lateralis will be obtained from the subjects to determine the fibre type distribution after ATPase staining. Mitochondrial capacity will be measured ex-vivo biochemically with high-resolution respirometry to examine oxidative capacity of the muscle tissue. After 12 weeks, the initial tests and muscle biopsies will be repeated.

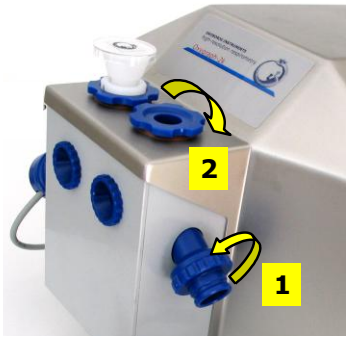
In conclusion, we want to collect additive data with different methods that can give insights into mechanisms contributing to adaptations of skeletal muscle. Comparison of ex-vivo and in-vivo MRS analysis will foster our understanding of skeletal muscle metabolism. Our model will permit to study and quantify biochemical and molecular processes associated with a change in muscle metabolism due to different external stimuli.

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)

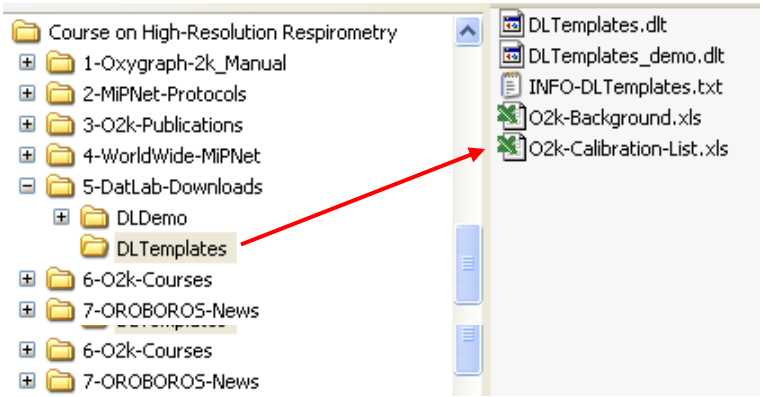
2.1. Why is it important to check the non-calibrated raw signal (voltage, after current-to-voltage conversion) of the polarographic oxygen sensor, and how can you quickly see the raw signal on-line?
2.2. The sensor voltage is above 9.9 V. What should you do?
2.3. Why is it important to maintain an extremely 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. When and how frequently is a POS service required?
4.2. What should be done if the sensor connector threads appear dark and dirty?
4.3. The POS membrane box appears to have two types of membranes, which one should be applied to the sensor?
4.4. How can you avoid creating bubbles when filling the electrolyte reservoir of the POS?
4.5. Can the ammonia treatment be applied repeatedly?
4.6. How can you check sensor performance?
4.7. 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?

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. **WorldWide-MiPNet: Mitochondrial Physiology Network**

Accomodation and Location

Hotel Körbersee www.koerbersee.at;
Tel +43 5519 265; hotel@koerbersee.at

Early Adventures at Lakes Kalbelesee and Körbersee

Gnaiger E (1993) Adaptations to winter hypoxia in a shallow alpine lake. Ecophysiological energetics of *Cyclops abyssorum* and rainbow trout. *Verh. Dtsch. Zool. Ges.* 86: 43-65.

Ecophysiology at Lakes Kalbelesee and Körbersee, 1974-1980

IOC56. *Mitochondrial Physiology Network* 15.2: 10-16 (2010)
Last update: 2010-03-30

O2k-MultiSensor Workshop: From TPP^+ to mt-Membrane Potential and Respiratory States

2nd O2k-MultiSensor Workshop

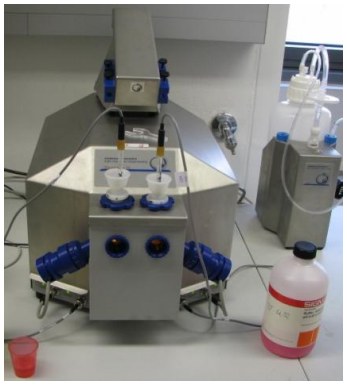
2010 April 12 – 15

Schröcken, Vorarlberg, Austria

The 56th IOC presents the newest developments of the **MultiSensor O2k-MiPNetAnalyzer** with the OROBOROS Ion Selective Electrode (ISE) for simultaneous monitoring of respiration and TPP^+. Futher MultiSensor perspectives will be discussed shortly (pH, NO, spectrophotometry, spectrofluorimetry). Exchange of expertise of all participants will be as important as the contributions of our partner companies and the organizers.

Partner companies

- SAFAS, Monaco: Xenius Spectrofluorimeter-spectrophotometer and optical fibre light guide into the O2k chamber.
- LEA Medizintechnik, Germany: O2c Spectrophotometer and optical fibre light guide into the O2k chamber.



MultiSensor applications imply an increased complexity of experimental design. Additional sensors or light guides inserted through the stopper into the O2k-chamber may compromise some features of high-resolution respirometry: The optimum volume is 2 ml with the TPP^+ electrode or NO sensor, or 3 ml with the pH electrode. The lower sensitivity of some electrodes compared to the oxygen measurement requires higher sample concentrations. Oxygen backdiffusion may be increased. Electrodes and light guides extending into the O2k-chamber increase the difficulty of removing gas bubbles. Accessibility of the titration port of the stopper is restricted, requiring elongated needles of the titration syringes. The difficulties will be addressed in the O2k-MultiSensor Workshop, and solutions are presented in theory and practice.

Guest Lecturer

- **Borutaite Vilma,** PhD, Kaunas University of Medicine, LT

Lecturers & Tutors

- **Erich Gnaiger,** PhD, OROBOROS INSTRUMENTS, AT
- **Kathrin Renner-Sattler**, PhD, OROBOROS INSTRUMENTS, AT
- **Mario Fasching,** PhD, OROBOROS INSTRUMENTS, AT
- **Zuzana Sumbalová,** PhD, OROBOROS INSTRUMENTS, AT
- **Anita Wiethüchter**, MSc, OROBOROS INSTRUMENTS, AT *admin.*

Programme IOC56

Day 1: Monday, 12. April

15:00 Participants arriving in Bregenz: Meeting point at 3:00 pm in Bregenz train station; 1.1 hour drive to Schröcken. Transfer and check in at Hotel Körbersee.

18:30 Welcome reception

19:00 Dinner

21:00-21:20 Erich Gnaiger (*Innsbruck, AT*) Expectations and reality in MutiSensor high-resolution respirometry.

21:20-22:00 Introductions of participants and their research interests.

Day 2: Tuesday, 13. April

8:30 – 10:00 **Mario Fasching, Erich Gnaiger, Kathrin Renner-Sattler: Introduction to MultiSensor methodologies and applications with permeabilized cells.** The TPP^+ electrode – an example for ion selective electrodes.

10:00 Coffee

10:30 – 12:00 **Demo experiment: Calibration of the TPP^+ electrode, and instrumental oxygen background in the presence of additional sensors**

12:00 - 14:30 Lunch break

14:30 -18:30 **Parallel group sessions:** Hands-on O2k and TPP^+

19:00 Dinner

Hot topics: MiPNet Session (10+10 min)
Chair: Vilma Borutaite, Kathrin Renner-Sattler

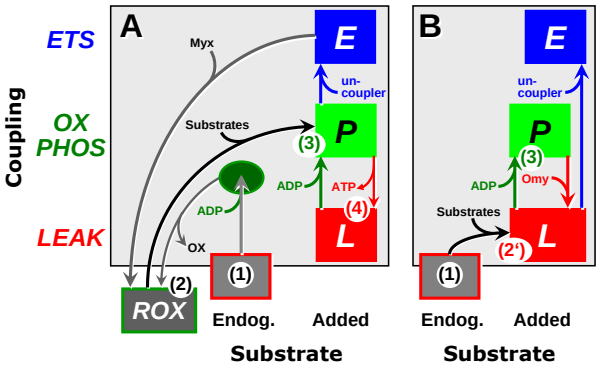
21:00 - 21:20 **MiPNet 1: Votyakova Tatyana** (*USA*) Effect of external and matrix proton concentration in ROS production by mitochondrial respiratory chain.

21:20 - 21:40 **MiPNet 2: Eckert Schamim** (*Germany*) Dimebon protects against complex I induced mitochondrial dysfunction.

21:40 - 22:00 **MiPNet 3: Lanza Ian** (*USA*) Caloric restriction attenuates many age-related changes in skeletal muscle mitochondrial physiology.

Day 3: Wednesday, 14. April

08:30 – 10:00 **Erich Gnaiger (*Innsbruck, AT*) Metabolic steady states: from cytochrome redox states and respiration to mt-membrane potential**. Beyond State 3 and 4.



10:00 Coffee

10:30 – 12:00 **Vilma Borutaite (Kaunas, Lithuania): Ion distribution and mitochondrial membrane potential – theory.**

12:00 – 14:00 Lunch break

14:00 -16:30 **Mario Fasching, Zuzana Sumbalová: From the TPP^+ signal to mitochondrial membrane potential. DatLab 4.3 and DatLab-Excel analysis templates.**

16:30 Coffee

17:00 – 18:45 **Open topics:**

A. Workpackage pH: setup of O2k with pH electrodes, volume calibration demo, pH calibration, electrode maintenance; application of the pH electrode: pH measurements in weak buffer, buffering capacity and experimental design.

B. Workpackage NO: electrode assembly, maintenance, calibration of the ion selective electrode; open chamber electrode calibration; blank experiment, data analysis.

C: Workpackage Oxygen kinetics and steady state experiments

19:00 Dinner

21:00 ***IOC56 feedback - discussion - summary – conclusions***
Farewell party of IOC56

Day 4: Thursday, 15. April

Early morning: Departure

MiPNet Abstracts–

Hot topics in Mitochondrial Physiology

MiPNet 56.1. Effect of external and matrix proton concentration in ROS production by mitochondrial respiratory chain

Selivanov VA[1, 2], Zeak JA[3], Roca J[4], Cascante M[1], Trucco M[3], Votyakova TV[3]

[1] Department of Biochemistry and Molecular Biology, University of Barcelona (IBUB) Barcelona, Spain. [2]A.N.Belozersky Institute of Physico-Chemical Biology, Moscow State University, Moscow, Russia. [3]Department of Pediatrics, the University of Pittsburgh School of Medicine, Children's Hospital of Pittsburgh, Pittsburgh, Pennsylvania, USA. [4]Hospital Clínic, IDIBAPS, University of Barcelona, Barcelona, Spain.

Reactive oxygen species (ROS) generation in mitochondria is a side-product of electron and proton transport through the inner membrane. It is important for normal cell operation as well as development of a number of pathologies. Matrix and cytosol alkalization stabilizes semiquinone radical and we hypothesized that proton deficiency under the excess of electron donors enhances reactive oxygen species generation. We tested this hypothesis by measuring pH dependency of reactive oxygen species released by mitochondria. The experiments were performed in the media with pH varying from 6 to 8 in the presence of complex II substrate succinate, or under more physiological

conditions with complex I substrates glutamate and malate. Matrix pH was manipulated by inorganic phosphate, nigericine, and low concentrations of uncoupler or valinomycin. We found that high pH strongly increased the rate of free radical generation in all the conditions studied, even when ΔpH=0 in the presence of nigericin. In the absence of inorganic phosphate, when the matrix was the most alkali, pH shift in the medium above 7 induced permeability transition accompanied by the decrease of ROS production. ROS production increase induced by the alkalization of medium was observed with intact respiring mitochondria as well as in the presence of complex I inhibitor rotenone which enhanced reactive oxygen species release. The phenomena revealed in this report are important for understanding mechanisms governing mitochondrial production of reactive oxygen species, in particular that related with uncoupling proteins.

MiPNet 56.2. Dimebon protects against complex I induced mitochondrial dysfunction

Schamim H Eckert, Kristina Leuner, Walter E Müller
Goethe-University, Dept. of pharmacology, Biocenter, Frankfurt, Germany

The efficacy of dimebon, an old russian antihistaminic drug was recently investigated in AD patients. Doody *et al.* [1] compared the effects of dimebon in AD patients against placebo over six months and measured cognitive and non-cognitive outcomes with the ADAS-cog scale and the neuropsychiatric inventory scale (NPI). Dimebon was significantly superior to placebo in all investigated parameters. There are different findings that dimebon moderately inhibits cholinesterases, blocks NMDA receptor signalling, inhibits the mitochondrial permeability transition pore opening, stabilizes the mitochondrial membrane potential (MMP) and induces neurite outgrowth. However, the molecular target of dimebon is still not known and the evidence of dimebon's effects on mitochondrial function is highly discussed. Therefore, we investigated if dimebon protects mitochondria against complex I mediated mitochondrial dysfunction which also plays a major role in aging and Alzheimer disease [2]. We used the complex I inhibitor rotenone and investigated the effects of dimebon on mitochondrial function, morphology and on ATP-levels after rotenone induced mitochondrial stress in HEK cells and PC12 cells. We chose three different treatment regimes: 24 h pretreatment, 1 h pretreatment and 1 h posttreatment with Dimebon 100 nM. Afterwards, cells were stressed for 6 h with rotenone 25 µM. Dimebon significantly protected MMP and ATP levels after 1 h pretreatment in HEK cells and PC12 cells. However, the effects were rather moderate. Therefore, we also investigated the effect of dimebon on mitochondrial morphology in HEK cells using confocal laser scanning microscopy and mito CMX-ROS because this parameter is more sensitive compared to MMP and ATP levels. In the control cells, a high density of mitochondria with long-tubular shape was detected. In cells stressed with rotenone mitochondria fragmented, showed punctuate morphology and cumulate around the nucleus. Dimebon preincubation highly compensated against rotenone induced mitochondrial fragmentation. Mitochondria again show longer and tubular shape such as in control cells.

Conclusion: Dimebon protects MMP and ATP levels after complex I induced mitochondrial dysfunction. In addition, mitochondrial morphology is restored to control levels under the treatment with dimebon Hence dimebon shows direct effects on mitochondrial function and represents a promising new therapeutic drug against mitochondrial dysfunction in neurodegenerative diseases.

[1] Doody RS et al (2008) Effect of dimebon on cognition, activities of daily living, behaviour, and global function in patients with mild-to-moderate Alzheimer´s disease: a randomised, double-blind, placebo-controlled study. Lancet 372: 207-215.
[2] Hauptmann S et al (2009) Mitochondrial dysfunction: An early event in Alzheimer pathology accumulates with age in AD transgenic mice Neurobiology of Aging 30: 1574–1586.

Participants and Areas of Interest (IOC55/56)

Bliksøen Marte, PhD, IEMR Oslo University Hospital, Norway. martbl@medisin.uio.no (*ischemia reperfusion injury, heart, gender differences*) - IOC55

Borutaite Vilma, Prof. PhD, Institute for Biomedical Research, Kaunas University of Medicine, Lithuania. vilbor@vector.kmu.lt (*invited speaker*) - IOC56

Burgstaller Wolfgang, Prof. PhD, Institute of Microbiology, University of Innsbruck, Austria. Wolfgang.Burgstaller@uibk.ac.at (*alternative respiration in filamentous fungi*) - IOC55

Contreras Karla, PhD, Monash University, Division of Biological Engineering, Clayton, Australia. kgcon2@student.monash.edu (*respiration in stem cells and mammalian cells*) - IOC55

Cortez Erika, PhD, Laboratory of Physiology of Nutrition and Development, Department of Physiological Sciences Federal University of Rio de Janeiro, Brazil. cortez.erika@gmail.com (*changes in energy metabolism of lymphocytes as biomarkers of nutritional interventions effects*) - IOC55/56

Davies Stefan, PhD, University of Western Australia, Institute for Medical Research, Perth, Australia. stefan.davies@waimr.uwa.edu.au (*role of new nuclear-encoded mt proteins in cells and isolated mitochondria; bioenergetics, mt gene expression*) - IOC55/56

Dzialowski M. Edward, PhD, Department of Biological Sciences, University of North Texas, Denton, USA. ed.dzialowski@unt.edu *(developmental changes in mitochondria function)* - IOC56

Eckert Schamim, Institute of Pharmacology, Frankfurt, Germany. s.haidari@em.uni-frankfurt.de (*mt dysfunction in neurodegenerative diseases; investigating the function of the respiratory chain complexes – with and without challenge – by the O_2-consumption*) - IOC56

Fasching Mario, PhD, OROBOROS INSTRUMENTS, Innsbruck, Austria. mario.fasching@oroboros.at (*lecturer, tutor*) - IOC56

Flynn Robb, PhD, Vanderbilt University Medical Center, Department of Surgical Sciences, Nashville, USA. robb.flynn@vanderbilt.edu (*non-alcoholic fatty liver disease; altered expression of mt proteins involved with fat oxidation and accumulation of lipid oxidation intermediates implicating multiple impairments in mt function)* - IOC55

Galina Antonio, Prof. PhD, Institute of Medical Biochemistry, Federal University of Rio de Janeiro, Brazil. galina@bioqmed.ufrj.br (*multisensor of membrane potential of $\Delta\Psi$, NO, pH and O_2*) - IOC56

Gnaiger Erich, A.Univ.-Prof. PhD, D. Swarovski Research Laboratory, Dept. General Transplant Surgery, Medical University Innsbruck; OROBOROS INSTRUMENTS; Austria. erich.gnaiger@oroboros.at *(organizer, tutor)* - IOC55/56

Gonçalves Renata de Lima Sales, PhD, Institute of Biomedical Medicine, Federal University of Rio de Janeiro, Brazil. rlsales@bioqmed.ufrj.br (*linking the mt metabolism and mosquito immune response, pointing the regulation of mt ROS as an effective immune tool for A. gambiae against Plasmodium infection; mitochondria, flight muscle, vector disease and hematophagous*) - IOC55/56

Habets Daphna, Department of Clinical Genetics, Maastricht University Hospital, Netherlands. d.habets@gen.unimaas.nl (*respiration in cultured fibroblasts and isolated mitochondria of patients with defects in the mt respiratory chain; diagnostics, metabolic inborn error*) - IOC55

Jacobs Robert, Institute of Veterinary Physiology, University of Zurich, Switzerland. jacobs@vetphys.uzh.ch (*regulation of mt respiration in response to hypoxia and/or exercise in skeletal muscle and adipose tissue; hypoxia, oxidative capacity, mt biogenesis, skeletal muscle, adipose tissue*) - IOC55

Janke Linda, PhD, German Diabetes Center, Leibniz Center for Diabetes Research at Heinrich Heine University, Düsseldorf, Germany. Linda.Janke@ddz.uni-duesseldorf.de (*comparison of type 1 diabetes and type 2 diabetes in mice; interaction between insulin resistance and mitofunction, diabetes, permeabilized muscle fibers, mouse models*) - IOC55/56

Kuncová Jitka, PhD, Faculty of Medicine in Plzen, Charles University in Prague, Czech Republic. Jitka.Kuncova@lfp.cuni.cz (*mt function in sepsis and chronic renal failure*) - IOC56

Labieniec Magdalena, PhD, Department of General Biophysics, University of Lodz, Poland. magdalab@bio.uni.lodz.pl - IOC55

Lanza Ian R., PhD, Mayo Clinic, Rochester, USA. lanza.Ian@mayo.edu (*mt function in aging, exercise and nutrition*) - IOC56

Larsen Filip, PhD, Karolinska Institute, Swedish School of Sport and Health Sciences, Stockholm, Sweden. filip.larsen@ki.se (*nitric oxide, bioenergetics, exercise physiology)* - IOC56

Lundby Carsten, Prof. PhD, Center for Integrative Human Physiology, University of Zurich, Switzerland. carsten.lundby@access.uzh.ch - IOC55

Paglialunga Sabina, Department of Human Biology, Maastricht University, The Netherlands. S.paglialunga@hb.unimaas.nl - IOC55/56

Pesta Dominik, Mag, D. Swarovski Research Laboratory, Dept. General Transplant Surgery, Medical University Innsbruck; and OROBOROS INSTRUMENTS; Austria. dominik.pesta@student.uibk.ac.at *(tutor)*

Phielix Esther, German Diabetes Center, Heinrich-Heine-University Düsseldorf, Germany. esther.phielix@ddz.uni-duesseldorf.de (*tutor*) - IOC55; (*membrane potential and ROS production in permeabilized muscle fibres*) - IOC56

Rae Epp, PhD, University of Tartu, Estonia. eppu_ra@yahoo.com (*experiments with skinned cardiac fibers at different oxygen level)* - IOC55

Rasmussen Peter, PhD, Center for Integrative Human Physiology, University of Zurich, Switzerland. peter@prec.dk - IOC55

Renner-Sattler Kathrin, PhD, OROBOROS MiPNet, Regensburg, Germany. kathrin.renner@oroboros.at (*lecturer, tutor*) - IOC55/56

Salvadego Desy, Department of Biomedical Sciences and Technologies, University of Udine, Italy. desy.salvadego@uniud.it (*peripheral limitations to oxidative metabolism in body builders; maximal oxygen consumption, mt activity, knee-extension exercise*) - IOC55

Schiffer Tomas, PhD, Karolinska Institute, Swedish School of Sport and Health Sciences, Stockholm, Sweden. tomas.schiffer@ki.se (*nitric oxide, bioenergetics, exercise physiology*) - IOC56

Schneider Maren, Cell Biology & Molecular Aging Research, Environmental Health Research Institute at the Heinrich-Heine-University, Duesseldorf, Germany. maren-schneider@t-online.de (*respiratory measurements after induced stress in cultured cells*) - IOC55

Schreilechner Anna, PhD, Department of Medical Biochemistry & Medical Molecular Biology, University of Graz, Austria. anna.schreilechner@meduni-graz.at (*respiratory oxygen consumption in cells and tissue and isolated mitochondria*) - IOC55

Senft Katharina, University of Cologne, CECAD Cologne-Excellent in Aging Research at the Institute for Genetics, Cologne, Germany. katharina.senft@uk-koeln.de (*mt dysfunction in aging-associated diseases*) - IOC55

Siebenmann Christoph, PhD, Institute of Physiology, University of Zurich, Switzerland. csiebenm@student.ethz.ch - IOC55

Stensløkken Kåre-Olav, Post Doc, Institute of Molecular Biosciences, University of Oslo, Norway. k.o.stenslokken@imbv.uio.no (*border between comparative physiology and medical physiology; heart and ischemia reperfusion injury in mammalian hearts and the study of an anoxia tolerant fish, the crucian carp*) - IOC55

Stepto Nigel, PhD, School of Sports and Exercise Science, Centre for Aging, Rehabilitation, Exercise and Sport (CARES), Melbourne, Australia. nigel.stepto@vu.edu.au (*mt function in skeletal muscle in humans; role of adaptation to exercise for health and performance; polycystic ovary syndrome, insulin resistance, women´s health; training and altitude interventions*) - IOC55/56

Sumbalová Zuzana, PhD, OROBOROS INSTRUMENTS, Innsbruck, Austria. zuzana.sumbalova@oroboros.at (*tutor*) - IOC55/56

Szendrödi Julia, PhD, German Diabetes Center, Leibniz Center for Diabetes Research at Heinrich Heine University, Düsseldorf, Germany. julia.szendroedi@ddz.uni-duesseldorf.de (*insulin resistance of mitochondria*) - IOC56

Van Bergen Nicole, Glaucoma Research Laboratory, Centre for Eye Research Australia, Melbourne, Australia. nicolevb@unimelb.edu.au (*mt dysfunction in cultured cells and isolated mitochondria of patients with defects in the mt respiratory chain, mt dysfunction in aging, mt biogenesis; mitochondrial dysfunction in optic neuropathies*) - IOC55/56

Vieira Beiral Hellen Jan, PhD, Department of Biophysics and Physiology, Federal University of Rio de Janeiro, Brazil. hellen.jan@biof.ufrj.br - IOC55/56

Votyakova Tatyana, PhD, Children's Hospital of Pittsburgh of UPMC, USA. tav2@pitt.edu (*basic mechanisms of ROS generation by mitochondria and mt mechanisms related to the pathologies of diabetes; monitoring respiratory activities of isolated beta-cells from various sources in order to relate this parameter with the cells wellbeing and suitability for transplantation*) - IOC56

Vrabl Pamela, PhD, University of Innsbruck, Institute of Microbiology, Austria. pamela.vrabl@uibk.ac.at (*alternative respiration in filamentous fungi*) - IOC55

Watala Cezary, Prof. PhD, Department of Haemostasis and Haemostatic Disorders, Medical University of Lodz, Poland. cwatala@csk.umed.lodz.pl - IOC55

Wetterwald Céline, PhD, Lab Biochim, Centre Hospitalier Univ. d'Angers, France. sunja@hotmail.fr (*mt pathologies: biochemical diagnosis, research on mt neuropathies; development of complementary analyses using TPP^+, Ca^{2+} electrodes*) - IOC56

Wiethüchter Anita, MSc, OROBOROS INSTRUMENTS, Innsbruck, Austria. anita.wiethuecher@oroboros.at (*scientific coordinator*) - IOC55/56

Contact

Erich Gnaiger, PhD
Medical University of Innsbruck
Department of Transplant Surgery
D. Swarovski Research Laboratory
Innrain 66/6
A-6020 Innsbruck, Austria
T +43 512 504 24623 (24626)
F +43 512 504 24625 20
Email erich.gnaiger@i-med.ac.at
www.mitophysiology.org