

139th O2k-Workshop on High-Resolution Respirometry

2019 Jun 17 – 22
Schroecken, Vorarlberg, Austria



The **139th O2k-Workshop on high-resolution respirometry (HRR)** is the **41st** International Oxygraph Course held in Schroecken since 1988. We provide an overview of the **O2k-FluoRespirometer**, with real-time analysis by **DatLab 7.4 (new)** and applications of the **Titration-Injection microPump TIP2k**. O2k-Demo experiments demonstrate the unique advantages and limitations of simultaneous monitoring of oxygen concentration, respiration, and hydrogen peroxide production. HEK 293T cells are used as a biological reference sample, which can be stored and shipped on dry-ice – introducing the MitoFit Proficiency Test.

Instrumental setup and service of the polarographic oxygen sensor (**OroboPOS**) are demonstrated, followed by hands-on practice in 10 teams. A wide range of mitochondrial topics is covered; abstracts and experimental experiences are presented by participants. IOC participants invariably asked for a detailed discussion of protocol design. The **Blue Book** (5th edition in prep.) and the **Mitochondrial respiratory states and rates** provide a basic introduction to mitochondrial physiology, complemented by overview presentations with examples, including **DatLab Analysis** of demo files. **Instrumental quality control** is a fundamental component of HRR and will be put to the practical test in teams using eight O2k (16 chambers). The **O2k-FluoRespirometer**, fully supporting **O2k-MultiSensor** applications, particularly fluorescence measurements, has become an integral part of the O2k-Workshop.

We present for the first time the **Oroboros SUITbrowser**. Many optimized SUIT protocols are now available in DatLab 7.4 as DL-Protocols. The SUITbrowser helps you to find the best SUIT protocol for your specific research questions.

Lunch breaks provide an opportunity for relaxing *Walks&Talks*, enjoying the refreshing scenery of the secluded alpine environment or using spare time for individual practice. Join for a visit to the *Alpmuseum*. secluded alpine

12:30	<i>Lunch packages/ Walk & Talk</i> <i>Alternative: individual O2k-tasks</i>	
14:30-15:30	Coupling control protocol and simultaneous measurement of H₂O₂ production in cryopreserved HEK cells (Demo-Experiment) DL-Protocol (O2&AmR): SUIT-003 AmR ce D017	SUIT-003 AmR ce D017
15:30	<i>Coffee / Tea</i>	
16:00-18:00	Hands-on (8 teams): Oxygen calibration and cell respiration Cell respiration and simultaneous measurement of H ₂ O ₂ production in cryopreserved living HEK cells DL-Protocol: O2 calibration air DL-Protocol (O2&AmR): O2k-cleaning AfterUse	Coupling control protocol SUIT-003 AmR ce D017
18:30	<i>Dinner</i>	
20:00-21:00	DatLab analysis: Quality control and reproducibility of technical repeats	DatLab-Analysis

3 Wednesday, Jun 19

Workshop 2		Weblink
07:30-08:30	<i>Breakfast</i>	
08:30-09:45	Experimental design: Pathway and coupling control of mitochondrial respiration	MitoPedia: Respiratory states
09:45-10:00	Oroboros SUITbrowser	Oroboros SUITbrowser
10:00	<i>Coffee / Tea</i>	
10:30-11:00	Substrate-uncoupler-inhibitor titration (SUIT) protocols – fundamental principles	MitoPedia: SUIT
11:00-11:30	O2k-Demo experiment: Respiration of permeabilized cells: Measurement of oxygen consumption with Reference protocols RP1 (SUIT-001) and RP2 (SUIT-002) DL-Protocol (O2): SUIT-001 O2 ce-pce D003 and SUIT-002 O2 ce-pce D007	SUIT reference protocol SUIT-001 O2 ce-pce D003 SUIT-002 O2 ce-pce D007
11:30-12:30	Hands-on (8 teams) - getting started with an O2k experiment: washing, stirrer test, air calibration DL-Protocol: O2k-cleaning BeforeUse DL-Protocol: O2 calibration air	SOP: O2k-cleaning and ISS SOP: POS-calibration
12:30	<i>Lunch packages / Walk & Talk</i> <i>alternative: individual O2k-tasks</i>	The Blue Book p 56*
14:00-16:30	Hands-on (8 teams) - O2k-experiment Respiration with permeabilized cells: SUIT protocols (RP1 and RP2) with 8 Power-O2k DL-Protocol (O2): SUIT-001 O2 ce-pce D003 and SUIT-002 O2 ce-pce D007 DL-Protocol: O2k-cleaning AfterUse	SUIT reference protocol SUIT-001 O2 ce-pce D003 SUIT-002 O2 ce-pce D007
16:00	<i>Coffee / Tea – Take turns in your team to continue the experiment</i>	
16:30-17:45	DatLab analysis and SUIT protocols Flux per volume, flux per mass, flow per cell, flux control ratio, flux control factor	MitoPedia: Respiratory control ratios MitoPedia: SUIT
17:45-18:45	DatLab analysis: hands-on in teams Analysis of the hands-on experiment with permeabilized cells.	O₂-Flux Analysis MitoPedia: DatLab
19:00	<i>Dinner + registration for the walk to the Alpmuseum</i>	
20:30-21:30	O2k perspectives: 10+5 min presentations of abstracts 1-4	

4 Thursday, Jun 20

Workshop 3		Weblink
07:30-08:30	<i>Breakfast</i>	
08:30-09:00	Introduction to H₂O₂ measurements	Amplex UltraRed H₂O₂
09:00-10:30	Hands-on (8 teams): Standard H₂O₂ protocol for permeabilized cells in 8 O2ks DL-Protocol (O2&AmR): SUIT-009 AmR ce-pce D019 DL-Protocol: O2k-cleaning AfterUse	Standard H₂O₂ protocol: AmR ce-pce D019
10:00	<i>Coffee / Tea – Take turns in your team to continue the experiment</i>	
10:30-11:00	Hands-on (8 teams): continuation DL-Protocol (O2&AmR): SUIT-009 AmR ce-pce D019 DL-Protocol: O2k-cleaning AfterUse	Standard H₂O₂ protocol: AmR ce-pce D019
11:00-12:30	H₂O₂ data analysis: introduction and hands-on in teams	
12:30	<i>Lunch packages / walk & talk alternative: individual O2k-tasks</i>	
14:30-15:50	DatLab analysis: hands-on in teams and summary discussion	O₂-Flux Analysis
15:50-16:30	From isolated mitochondria to tissue fibers and tissue homogenate preparation: The PBI-Shredder (overview with video clips)	MiPNet17.03 Shredder vs Fibres O2k-Videosupport
16:30	<i>Coffee / Tea</i>	
17:00-18:00	Data interpretation using SUIT protocols. OXPHOS analysis: diagnosis of respiratory defects	MitoPedia: SUIT Oroboros SUITbrowser
18:00-18:45	Introduction to mitochondrial membrane potential	Mitochondrial membrane potential
18:45-19:00	Introduction to analysis of mitochondrial oxygen kinetics	Oxygen kinetics
19:00	<i>Dinner</i>	
20:30-21:30	O2k perspectives: 10+5 min presentations of abstracts 5-9	

5 Friday, Jun 21

Workshop 4		Weblink
07:30-08:30	<i>Breakfast</i>	
08:30-09:00	Introduction to instrumental O₂ background (Demo-Experiment), using the TIP2k DL-Protocol: Instrumental O ₂ background TIP2k	SOP: O₂ background TIP2k manual
09:00-11:00	Hands-on (8 teams): Instrumental O₂ background (instrumental quality control 2) O ₂ background test with the TIP2k; analysis of oxygen flux; O ₂ background from air saturation to zero oxygen concentration; or for permeabilized muscle fibres in the high-oxygen range of 500 – 200 µM DL-Protocol: Instrumental O ₂ background TIP2k	SOP: O₂ background
10:30	<i>Coffee / Tea – Take turns in your team to continue the experiment</i>	MiPNet18.10 O2kvsMultiwell*
11:00-12:00	Data analysis	The Blue Book* pp 43-57
12:00	<i>Lunch packages</i>	
12:30-15:30	<i>Walk to the Alpmuseum - guided tour and reception: € 15.-</i>	Alpmuseum*
15:30	<i>Coffee / Tea</i>	
16:00-17:30	MitoFit Preprint Archives	MitoFit Preprint Archives O2k-Publications
17:30-18:15	Tutorial on the Bioblast wiki www.bioblast.at/	O2k-Network www.bioblast.at

18:30 *Dinner*20:00 *Feedback discussion: Next steps in the individual projects*

6 Saturday, Jun 22

Departure

06:30-7:30 *Breakfast*

Early morning: departure from Hotel Körbersee at 08:15 am, bus departure 9.00 am at Salober.

O2k-Workshop: OUR COMMON AIMS

- **Mitochondrial physiology:**
Study mitochondrial function in the **context** of cell physiology and pathology
- **Instrumental performance – the O2k:**
 - 🕒 Learn **High**-Resolution Fluorescence Respirometry
 - 🕒 Gain **hands-on** experience
 - 🕒 Extend to O2k-**Multi**Sensor applications
- **Excellence in research:**
 - 🕒 Instrumental **quality** control
 - 🕒 Experimental design for **innovation**
 - 🕒 Data analysis meeting superior **standards**

OROBOROS INSTRUMENTS

O2k

Mitochondria and cell research



List of participants

Participant	Institution
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*Asterisks indicate the number of O2k instruments in the participant's lab.

MiPNet24.01 Abstracts IOC139: 10+5 min O2k perspectives

1. **V Joseph, C Arias, J Soliz. (2019) Plasticity of brain mitochondrial respiration rates during acclimatization to chronic hypoxia in mice. Mitochondr Physiol Network 24.01.**

Several lines of evidence suggest that hypoxia decreases mitochondrial respiration rates, but some results show an opposite effect in rodents adapted to life at high altitude. In the present study we used FVB mice and SD rats, as two models showing divergent responses at high altitude or during short-term exposure to hypoxia at sea level. We assessed mitochondrial respiration rates in permeabilized brain cortex samples by high resolution respirometry (Oroboros-2k) during acclimatization to hypoxia. Rats and mice were exposed to room air (controls), short-term (6 and 24 hours), or long-term (7 and 21 days) hypoxia (12% O₂). Brain samples were rapidly dissected and permeabilized in saponin before measurements of O₂ consumption rates with a standard protocol to assess NADH-, FADH₂-, or NADH+FADH₂-linked respiration. During short-term hypoxic exposure, NADH and FADH₂-linked respiration increased in mice, but remained stable in rats. During long-term hypoxia in mice, while NADH-linked respiration remained elevated compared to controls, NADH+FADH₂-linked respiration returned to control levels while FADH₂-respiration decreased below control levels. Furthermore, in mice, H⁺ leak after rotenone and oligomycin (state 4 - FADH₂-linked) increased during short-term hypoxia, then declined during long-term hypoxia. None of these changes occurred in rats. Our results suggest specific plasticity of complexes I and II of the electron transport chain during

acclimatization to chronic hypoxia in mice, but not in rats. These divergent responses might contribute to a more efficient acclimatization to hypoxia in the central nervous system in mice.

2. Pamella Marie Sendon, Marie-Lune Simard, and James Stewart (2019) Development and Characterization of a Novel Mouse Model Carrying Specific Mitochondrial DNA Mutations. Mitochondr Physiol Network 24.01.

Mutations of the mitochondrial DNA (mtDNA) have long been of interest in the study of ageing and human diseases. These genetic mutations disrupt mitochondrial gene expression, leading to mitochondrial dysfunction. Unfortunately, it is still not possible to transgenically manipulate animal mtDNA so relevant animal models with mtDNA mutations are not abundant. To circumvent this, heterozygous female mutator mice, containing a proofreading-deficient form of mtDNA polymerase-gamma, were used in this study to transmit mtDNA mutations to the offspring. Lineages of nuclear-wild type mice transmitting acquired mtDNA mutations were then developed through series of breeding and screening. Histochemical analyses and sequencing were used to identify specific alleles that might play a role in mitochondrial dysfunction. Three SNPs were identified: a mutation in the mt-tRNAAla gene, a synonymous mutation in mt-cytb, and a mutation within the TFAM binding site of the mitochondrial control region. The mutations in mt-tRNAAla and mt-cytb do not have pathogenic effects, however, the mutation in the control region may affect mtDNA replication and transcription. Quantitative PCR results showed that mtDNA copy number in heart tissue of mutant mice was significantly lower compared to the WT; in the skeletal muscle, however, there was no significant difference. These results suggest that mtDNA replication is affected by the mutation in a tissue-specific manner. Steady state mt-mRNA levels in the heavy strand of heart tissue were lower in the mutant than the WT but no significant difference was observed in the light strand. To check for heteroplasmy, mutation levels between mothers and offspring were measured. At present, the highest mutation level is at 88% and the lowest is at 20%. Mutation levels across tissues were also measured, with high variability in colon and liver. Cardiomyopathy was observed in ageing mutant mice (61 to 90-week-old). Other experiments will be carried out, such as, in vitro replication and transcription assays, histochemical staining of other tissues, and comparison of mitochondrial respiration between WT and mutant mice to further molecularly characterize this new mouse model.

3. Montana R. Fulton and Michael S. Davis (2019) Effect of hyperthermia on mitochondrial function in canine skeletal muscle. Mitochondr Physiol Network 24.01.

Skeletal muscle can experience a 20-fold range of rate of metabolic heat production in athletes, subjecting mitochondria to levels of hyperthermia that likely alter oxidative phosphorylation (OxPhos). The exact nature of these alterations are likely temperature-dependent, as potential increases in enzyme activity are eventually offset by thermal damage. Specifically, we hypothesize that one result of increased temperature is an increase in proton leak across the mitochondrial membrane. However, well-conditioned athletes are known to be more tolerant to hyperthermia, but it is unknown whether this ability to preserve athletic function during heat stress is due to greater mitochondrial tolerance to hyperthermia. A previous study with racing sled dogs showed that at a single temperature of 37 °C, proton leak decreased as fitness levels increased. Thus, this study is part of a project to test the hypothesis that athletic conditioning results in greater mitochondrial OxPhos capacity at high temperatures.

Eight fully conditioned Alaskan sled dogs were used to evaluate the effect of ex vivo hyperthermia on skeletal muscle OxPhos. Skeletal muscle biopsies were obtained from the biceps femoris muscle after 7 months of progressive endurance conditioning that included competition in the annual 1000-mile Iditarod sled dog race. The biopsies were permeabilized and analyzed using high resolution respirometry at 4 different incubation temperatures (38, 40, 42, and 44°C) using standard SUIT protocols. Effect of incubation

temperature was analyzed using repeated measures one-way ANOVA and post-hoc pairwise comparisons using Tukey's correction for multiple comparisons.

Six of the eight dogs used in this study completed the 1000-mile race in 11 days, 10 hr (22nd place), with the other 2 dogs having completed approximately 300 miles before being removed from the team due to injury. Respirometry measurements for one dog was limited to PGML and PGMP due to technical problems during analysis. At 38°C (the normal body temperature of a dog), the respiratory states PGML, PGMP, PGMSP, and SP resulted in 29.4 ± 5.2 , 106.8 ± 47.1 , 184.4 ± 52.3 , and 100.6 ± 32.0 pmol/mg/sec of oxygen flux, respectively. PGML was strongly affected by temperature ($p < 0.0001$), with a maximum value of 49.5 ± 8.8 at 44°C. No other respiratory states were significantly affected by incubation temperature.

Data collected from this study show that hyperthermia induces spontaneous OXPHOS uncoupling in canine skeletal muscle. We cannot definitively say where proton leak is occurring at this moment and the implications of increasing proton leak during hyperthermia are not understood fully at this time, but it is known in other species that increased membrane potential is associated with hydrogen peroxide production and therefore leads to oxidative damage to the mitochondria. Concurrent mitochondrial membrane potential measurements could aid in discovering the consequences of proton leak on mitochondrial function. Studies are scheduled to repeat these measurements in the same dogs in a deconditioned state to further characterize the effects of athletic conditioning on temperature-dependent mitochondrial OxPhos.

K.M. O'Brien (2019) Mitochondrial form and function in Antarctic fishes. Mitochondr Physiol Network 24.01.

Oxidative and cardiac muscles of Antarctic icefishes, lacking the O₂-binding proteins hemoglobin and myoglobin, possess mitochondria markedly different in ultrastructure compared to red-blooded and red-hearted species. Mitochondria of icefishes are enlarged with lower cristae densities compared to red-blooded fishes (1). We hypothesized that the differences in ultrastructure would correlate with differences in function. However, despite their unusual mitochondrial architecture, the function of mitochondria from icefishes is similar to that of red-blooded species with state II and III respiration rates equivalent between the icefish, *Chaenocephalus aceratus* and the red-blooded species, *Notothenia coriiceps* (2). Most notable is the lower rate of cytochrome c oxidase activity (per mg mitochondrial protein) and absence of the mitochondrial sarcomeric isoform of creatine kinase (CK) in *C. aceratus* (3), which together likely contribute to their lower ATP levels per g tissue, and perhaps cardiac performance, compared to *N. coriiceps*. Questions remain about what regulates mitochondrial architecture, and how it is influenced by the expression of O₂- binding proteins in Antarctic fishes.

1. O'Brien KM, Sidell BD (2000) The interplay among cardiac ultrastructure, metabolism and the expression of oxygen-binding proteins in Antarctic fishes. *J Exp Biol* 203:1287-97.
2. O'Brien KM, Egginton SE, Farrell AP, Crockett EL, Schlauch K, Woolsey R, Hoffman M, Merriman S (2018) Cardiac mitochondrial metabolism may contribute to differences in thermal tolerance of red- and white-blooded Antarctic notothenioid fishes. *J. Exp. Biol.* 221.
3. O'Brien KM, Mueller IA, Orczewska JI, Dullen KR, Ortego M (2014) Hearts of some Antarctic fishes lack mitochondrial creatine kinase. *Comp Biochem Phys A* 178:30-6.

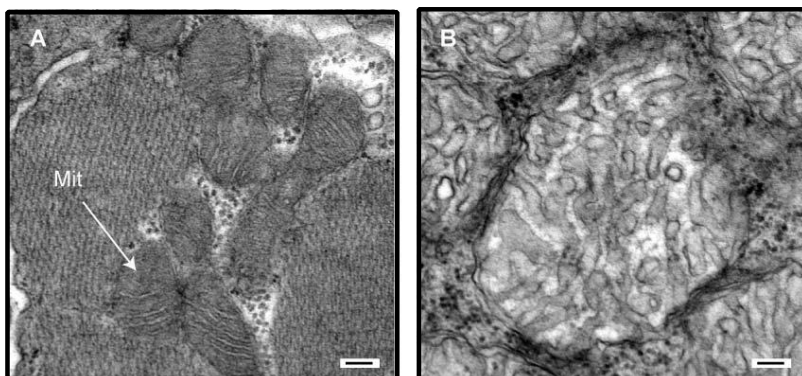


Figure 1: Transmission electron micrographs of mitochondria from heart ventricles of the red-blooded species, *N. coriiceps* (A) and icefish, *C. aceratus* (B). Magnification = 75,700 x, Bar = 100 nm.



MiPschool Coimbra 2019



Accommodation and location

Hotel Körbersee www.koerbersee.at
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More detail?

Gnaiger E (2019) Mitochondrial pathways and respiratory control. An introduction to OXPHOS analysis. 5th ed. Mitochondr Physiol Network 24.05. Oroboros MiPNet Publications, Innsbruck:96 pp. » [Full text in Bioblast](#)

O2k-Manual – <http://wiki.oroboros.at/index.php/O2k-Manual>

O2k-Procedures – <http://wiki.oroboros.at/index.php/O2k-Procedures>

>3,200 O2k-Publications – <http://wiki.oroboros.at/index.php/O2k-Publications: Topics>

COST Action CA15203 MitoEAGLE



MitoEAGLE Mitochondrial respiratory states and rates. Mitofit Preprint Arch doi:10.26124/mitofit:190001.v4
[Mitochondrial respiratory states and rates](#)

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Mitochondria and cell research

O2k-Workshops are listed as [MitoGlobal Events](#)

