Mitochondrial Physiology Network 24.01(02):1-10 (2019)
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Updates: http://wiki.oroboros.at/index.php/MiPNet24.01_IOC139_Schroecken_AT

139th O2k-Workshop on high-resolution respirometry

2019 Jun 17 – 22
Schröcken, 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 (*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** and the **Mitochondrial respiratory states and rates** provide a basic introduction to mitochondrial physiology and is 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. Optimization of protocol design for various O2k-MultiSensor applications helps to critically evaluate basic principles of mitochondrial physiology. You will also see the **TIP2k** with feedback-control in action and practice its simple and automatic operation.

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*.

Lecturers and tutors

Bastos Sant'Anna Silva Ana Carolina	PhD student TRANSMIT, Oroboros Instruments (AT)
Di Marcello Marco	Scientific Assistant, Oroboros Instruments (AT)
Doerrier Carolina	Chief scientific officer, Oroboros Instruments (AT)
Gnaiger Erich	CEO, Oroboros Instruments (AT)
Timea Komlodi	PostDoc, Oroboros Instruments (AT)
Passrugger Manuela	Biomedical assistant, Oroboros Instruments (AT)
Zdrazilova Lucie	Scientific researcher, Charles University (CZ)

Programme

1 Monday, Jun 17

*printed in workshop materials

	Arrival	Weblink
15:00	**Arrival in Bregenz:** Meeting point Bregenz train station at 3:00 pm; approx. 1 h bus drive to Schröcken and Hochtannberg (Salober); walk to Hotel Körbersee (approx. 40 min)	IOC-travel
18:30-19:30	*Welcome reception at Hotel Körbersee* & ***get-together:*** Introduction of participants and their research interests - a welcome by Oroboros Instruments	Schroecken
19:30	*Dinner*	

2 Tuesday, Jun 18

	Workshop 1		Weblink
07:30-08:30	*Breakfast*		
08:30-09:30	**Challenges of innovation and continuation: transition to O2k-Series H and DatLab 7** O2k instrumental setup – overview with video clips		O2k-FluoRespirometer MitoPedia: DatLab DL-Protocols O2k-Videosupport O2k-Start
09:30-11:30	**Hands-on (10 groups)**		
	DatLab 7	**OroboPOS service**	
09:30-10:15	Groups 1-5	Groups 6-10	POS Service
10:15	*Coffee / Tea*		
	DatLab 7	**OroboPOS service**	POS Service
10:45-11:30	Groups 6-10	Groups 1-5	O2k-Start
11:30-12:30	**Oxygen calibration (instrumental quality control 1)** DL-Protocol: O2k-cleaning BeforeUse DL-Protocol: O2 calibration air		Gnaiger 2008 POS SOP: O2-calibration
12:30	*Lunch packages/ Walk & Talk* *Alternative:* individual O2k-tasks		

14:30-15:30	**Cell respiration and simultaneous measurement of H_2O_2 production** (Demo-Experiment) DL-Protocol (O2&AmR): SUIT-013 AmR ce D023	O_2-Flux Analysis SUIT-013_AmR_ce_D023
15:30	*Coffee / Tea*	
16:00-18:00	**Hands-on (7 groups): Oxygen calibration and cell respiration** Cell respiration and simultaneous measurement of H_2O_2 production in intact cryopreserved HEK cells DL-Protocol: O2 calibration air DL-Protocol (O2&AmR): O2k-cleaning AfterUse	Coupling control protocol SUIT-013_AmR_ce_D023
18:30	*Dinner*	
20:00-21:00	**DatLab analysis:** Reproducibility of technical repeats	DatLab-Analysis

3 Wednesday, Jun 19

	Workshop 2	Weblink
07:30-08:30	*Breakfast*	
08:30-10:00	**Experimental design:** Pathway and coupling control of mitochondrial respiration	MitoPedia: Respiratory states
10:00	*Coffee / Tea*	
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 (7 groups) - 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: O2-calibration
12:30	*Lunch packages / Walk & Talk* *alternative:* individual O2k-tasks	*The Blue Book p 56**
14:00-16:30	**Hands-on (7 groups) - O2k-experiment** Respiration with permeabilized cells: SUIT protocols (RP1 and RP2) with 7 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	*Coffee / Tea - split team, continue with experiment*	
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.	O_2-Flux Analysis MitoPedia: DatLab
19:00	*Dinner + registration for the walk to the Alpmuseum*	
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	*Breakfast*	
08:30-10:30	**Hands-on (7 groups): Standard H_2O_2 protocol for permeabilized cells in 7 O2ks** DL-Protocol (O2&AmR): SUIT-009 AmR ce-pce D019 DL-Protocol: O2k-cleaning AfterUse	Standard H2O2 protocol: SUIT-009 AmR ce-pce D019
10:00	*Coffee/Tea - split team, continue with experiment*	

10:30-12:30	**H_2O_2 data analysis: introduction and hands-on in teams**	
12:30	*Lunch packages / walk & talk* *alternative:* individual O2k-tasks	
14:30-15:30	**DatLab analysis: summary discussion**	O_2-Flux Analysis
15:30-16:30	**From isolated mitochondria to tissue fibres and tissue homogenate preparation:** The PBI-Shredder (overview with video clips)	MiPNet17.03 Shredder vs Fibres O2k-Videosupport
16:30	*Coffee / Tea*	
17:00-18:00	**Data interpretation using SUIT protocols.** **OXPHOS analysis: diagnosis of respiratory defects**	MitoPedia: SUIT
18:00-19:00	**Introduction to analysis of mitochondrial oxygen kinetics and O2k**inetics **software**	
19:00	*Dinner*	
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	*Breakfast*	
08:30-09:00	**Introduction to instrumental O2 background** (Demo-Experiment), using the TIP2k DL-Protocol: Instrumental O2 background TIP2k	SOP: O2 background TIP2k manual
09:00-11:00	**Hands-on (7 groups): Instrumental O2 background (instrumental quality control 2)** O2 background test with the TIP2k; analysis of oxygen flux; O2 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 O2 background TIP2k	SOP: O2 background
10:30	*Coffee / Tea - split team, continue with experiment*	MiPNet18.10 O2kvsMultiwell*
11:00-12:00	**Data analysis**	The Blue Book* pp 43-57
12:00	*Lunch packages*	
12:30-15:30	*Walk to the Alpmuseum - guided tour and reception: € 15.-*	Alpmuseum*
15:30	*Coffee / Tea*	
16:00-17:30	**Data interpretation using O2k publications**	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 FluoRespirometry
  - 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**

Preliminary list of participants

Participant	Institution
Bach de Courtade Sandra Monica *	**NO Oslo Eide L** - Oslo University Hospital, Oslo (NO)
Bardal Tora **	**NO As Egelandsdal B** - Norwegian University of Science and Technology, Trondheim (NO)
Chabi Beatrice	**FR Montpellier Wrutniak-Cabello C** - INRA/University of Montpellier, Montpellier Cedex 1 (FR)
Cocco Paolo	**AT Innsbruck Oroboros** - Oroboros Instruments, Innsbruck (AT)
Davis Michael ****	**US OK Stillwater Davis MS** - Oklahoma State University, Stillwater (US)
Decker Stephen *	**US_MA Amherst_Busa MA** - University of Massachusetts Amherst, Amherst (US)
Di Paola Floriana Jessica	**TRANSMIT** student - Justus-Liebig-University of Giessen , Giessen (DE)
Fulton Montana ****	**US OK Stillwater Davis MS** - Oklahoma State University, Stillwater (US)
Grams Bente *	**DE Kronshagen Tillmans F** - Kiel University, Kronshagen (DE)
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Kwon Oh Sung*	**US CT Storrs Kwon O** - University of Connecticut, Storrs (US)
Leffler Märta ******	**SE Lund Elmer E** - Lund University, Lund (SE)
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Maull Felicia *	**DE Mainz Methner A** - Universitätsmedizin Mainz, Mainz (DE)
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Moore Christy *	**US TN Nashville Fessel JP** - Vanderbilt University Medical Center , Nashville (US)
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Roshanravan Baback *	**US CA Davis Roshanravan B** - University of California, Davis (US)
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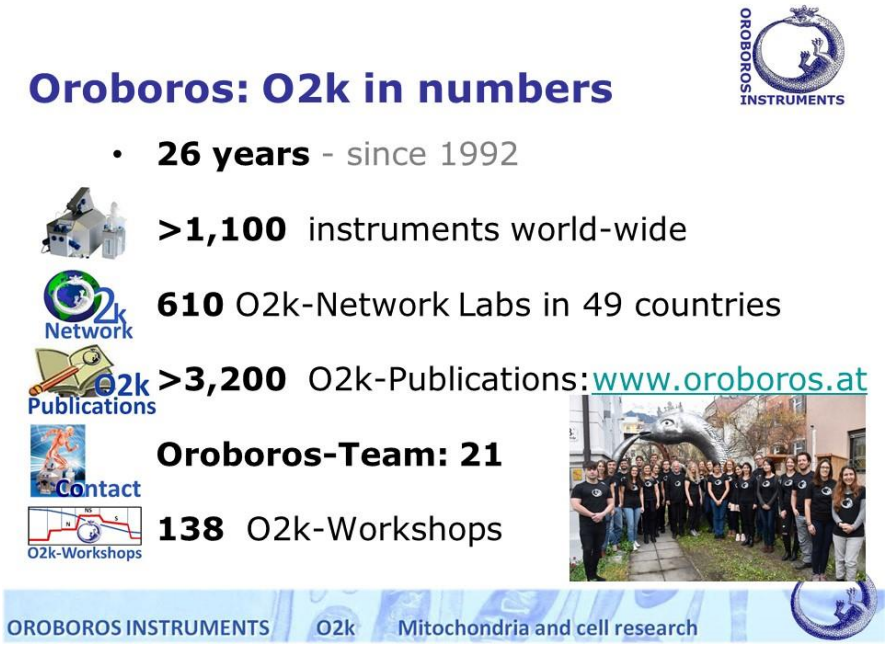
Sendon Pamella Marie	Max Planck Institute for Biology of Ageing, Cologne (DE)
Vlachaki Walker Julia *	**UK London Carling D** - MRC London Institute of Medical Sciences, London (UK)
Crossland Hannah*	**UK Exeter Blackwell JR** - University of Nottingham, Nottingham (UK)

*Asteriks 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, PGMSE, and SE 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, K. M., and Sidell, B. D. (2000) The interplay among cardiac ultrastructure, metabolism and the expression of oxygen-binding proteins in Antarctic fishes. *J Exp Biol* **203**, 1287-1297
2. O'Brien, K. M., Egginton, S. E., Farrell, A. P., Crockett, E. L., Schlauch, K., Woolsey, R., Hoffman, M., and 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, K. M., Mueller, I. A., Orczewska, J. I., Dullen, K. R., and Ortego, M. (2014) Hearts of some Antarctic fishes lack mitochondrial creatine kinase. *Comp Biochem Phys A* **178**, 30-36

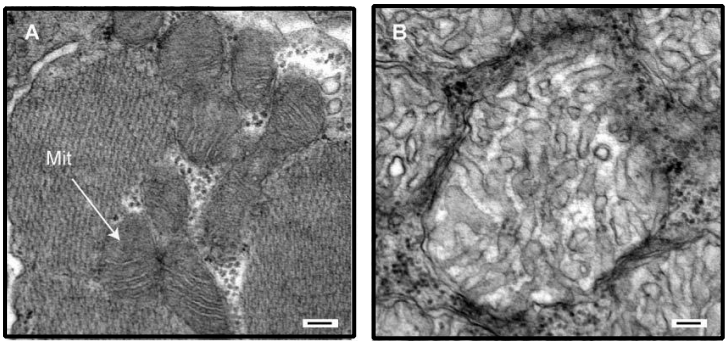


Figure legend: Transmission electron micrographs of mitochondria from heart ventricles of the red-blooded species, *N. coriiiceps* (A) and icefish, *C. aceratus* (B). Magnification = 75,700X, Bar = 100 nm.

Accommodation and location

Hotel Körbersee www.koerbersee.at
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More detail?

Gnaiger E (2014) Mitochondrial pathways and respiratory control. An introduction to OXPHOS analysis. 4th ed. Mitochondr Physiol Network 19.12. Oroboros MiPNet Publications, Innsbruck: 80 pp. » Full text in Bioblast

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

COST Action CA15203 MitoEAGLE

MitoEAGLE Mitochondrial respiratory states and rates.
doi:10.26124/mitofit:190001
Mitochondrial respiratory states and rates: Building blocks of mitochondrial physiology

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Mitochondria and cell research

O2k-Workshops are listed as MitoGlobal Events