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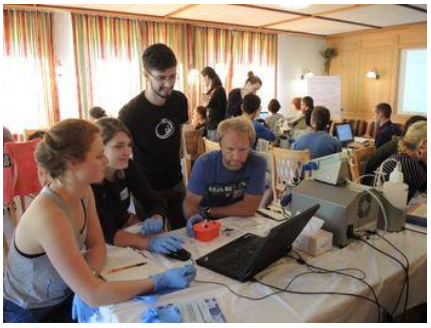
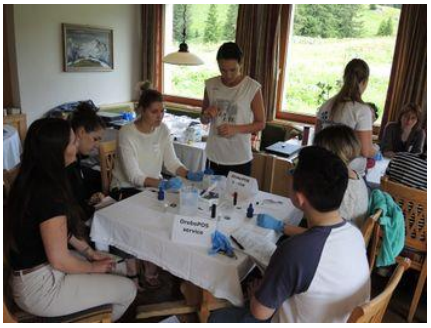
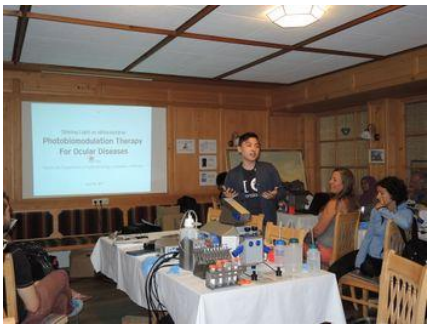
Updates: http://wiki.oroboros.at/index.php/MiPNet23.06_IOC130_Schroecken_AT

130th International Workshop on High-Resolution FluoRespirometry

2018 June 18 - June 23
Schröcken, Vorarlberg, Austria







The **130th Workshop on High-Resolution FluoRespirometry (HRFR)** is the **39th** 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** provides 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 HRFR and will be put to the practical test in teams using seven O2k (14 chambers). The **O2k-MultiSensor** and particularly O2k-Fluorometry 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

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Programme

1 Monday, Jun 18

*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 19

	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
09:30-11:30	**Hands-on (10 groups)**		O2k-Start
	O2k instrumental setup	**OroboPOS service**	
09:30-10:15	Groups 1-5	Groups 6-10	POS Service
10:15	*Coffee / Tea*		
	O2k instrumental setup	**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: 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 6	O_2-Flux Analysis SUIT 6
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): SUIT 6 DL-Protocol: O2k-cleaning after use	Coupling control protocol SUIT 6
18:30	*Dinner*	
20:00-21:00	**DatLab analysis:** Reproducibility of technical repeats	DatLab-Analysis

3 Wednesday, Jun 20

	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 1) and RP2 (SUIT 2) DL-Protocol (O2): SUIT 1 and SUIT 2	SUIT reference protocol
11:30-12:30	**Hands-on (7 groups) - getting started with an O2k experiment:** washing, stirrer test, air calibration DL-Protocol: O2k-cleaning before use 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**
14:00-16:00	**Hands-on (7 groups) - O2k-experiment** Respiration with permeabilized cells: SUIT protocols (RP1 and RP2) with 7 Power-O2k DL-Protocol (O2): SUIT 1 and SUIT 2 DL-Protocol: O2k-cleaning after use	SUIT reference protocol
16:00	*Coffee / Tea*	
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 21

	Workshop 3	Weblink
07:30-08:30	*Breakfast*	
08:30-09:00	**From isolated mitochondria to tissue fibres and tissue homogenate preparation:** The PBI-Shredder (overview with video clips)	MiPNet17.03 Shredder vs Fibres O2k-Videosupport
09:00-10:30	**Hands-on (7 groups): Standard H_2O_2 protocol for permeabilized cells in 7 O2ks** DL-Protocol (O2&AmR): SUIT 9 DL-Protocol: O2k-cleaning after use	Standard H2O2 protocol: SUIT 9

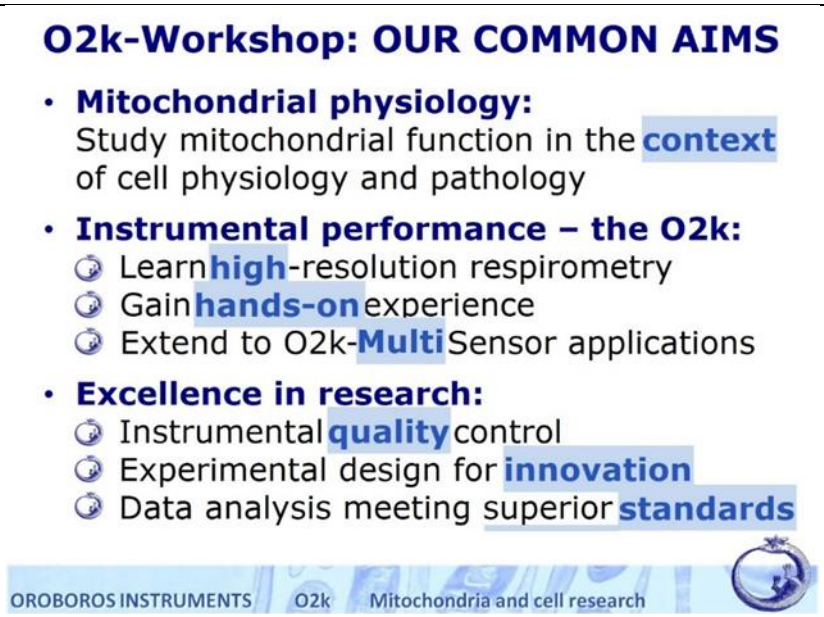
10:30	*Coffee / Tea*	
11:00-12:00	**H_2O_2 data analysis: introduction**	The Blue Book* pp 43-57
12:00	*Lunch packages / walk & talk* *alternative:* individual O2k-tasks	
14:30-15:30	**DatLab analysis: hands-on in teams**	O_2-Flux Analysis
15:30-16:00	**DatLab analysis: summary discussion**	
16:00	*Coffee / Tea*	
16:30-17:30	**Data interpretation using SUIT protocols. OXPHOS analysis: diagnosis of respiratory defects**	MitoPedia: SUIT
17:30-18:00	**Introduction to analysis of mitochondrial oxygen kinetics and O2k**inetics **software**	
18:30	*Dinner*	
20:00-21:15	**O2k perspectives:** 10+5 min presentations of abstracts 5-9	

5 Friday, Jun 22

	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
09:00-10:30	**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*	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*	
16:00-17:00	**Working groups: elaborate answers to the 'Questions for the O2k-Workshop'** - *come prepared*	IOC-Questions*
17:00-17:45	**IOC-questions - discussion of 'Answers', introduction to O2k-technical support**	O2k-technical support
17:50-18:45	**Tutorial on the Bioblast wiki** www.bioblast.at	O2k-Network www.bioblast.at
19:00	*Dinner*	
20:00	*Feedback discussion: Next steps in the individual projects*	

6 Saturday, Jun 23

	Departure
06:30-7:30	*Breakfast*
	Early morning: departure from Hotel Körbersee at 08:15 am, bus departure 9.00 am at Salober.



Participants

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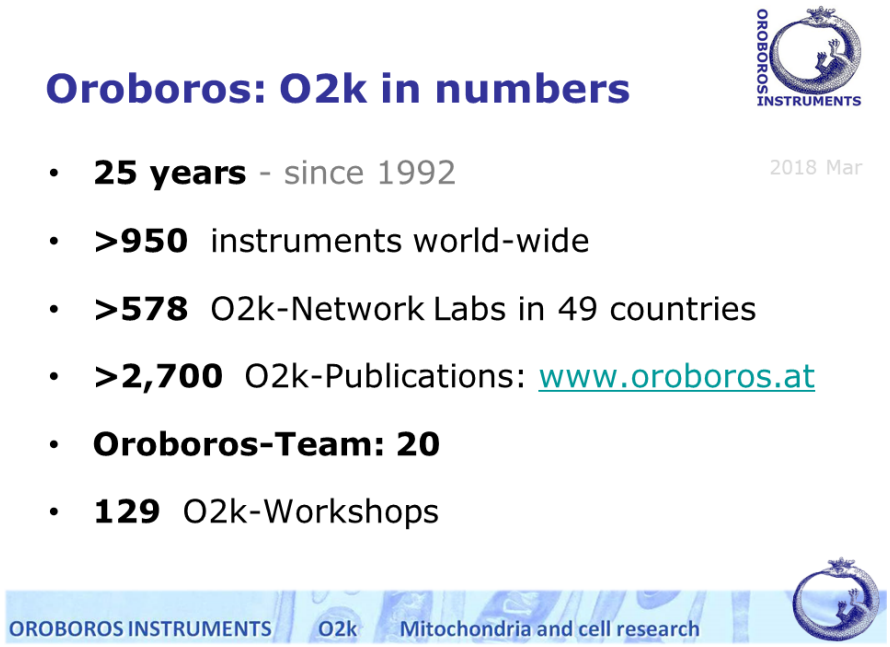
Zhang Feiyuan	**AT_Innsbruck_Oroboros:** Oroboros Instruments (AT)

*Asteriks indicate the number of O2k instruments in the participant's lab.



MiPNet23.06 Abstracts IOC130: 10+5 min

O2k perspectives

1. **Andriessen C (2018) Day-night rhythm in skeletal muscle metabolism of prediabetic men. Mitochondr Physiol Network 23.06.**

Modern life is characterized by a 24-hours mentality in which people's eating and sleeping behavior does not necessarily depend on the natural day and night rhythm (circadian rhythm). Both epidemiological- and intervention studies suggest that a disturbed circadian rhythm impairs metabolic health [1,2]. Indeed, a previous study showed that skeletal muscle oxidative phosphorylation in young lean males follows a circadian pattern [3]. However, it is currently not known if this pattern is disturbed in people with compromised metabolic health.

Overweight (BMI 25 – 35 kg/m2), prediabetic males, aged 40 – 70 years with a normal sleep-wake rhythm will be recruited for this observational study (n = 14). Participants will stay at the research unit for 44 hours, with standardized meals and sleeping time. Several measurements will be performed during this stay, including five muscle biopsies, indirect calorimetry using the ventilated hood, and several blood draws. Muscle biopsies will be used to assess skeletal muscle oxidative phosphorylation using High-Resolution FluoRespirometry.

2. **Nollet E (2018) Mitochondrial dysfunction in hypertrophic cardiomyopathy. Mitochondr Physiol Network 23.06.**

Hypertrophic cardiomyopathy (HCM) is a genetic cardiac disease, typified by left ventricular hypertrophy, diastolic dysfunction, myocyte disarray and increased risk of sudden cardiac death. HCM is the most common inherited cardiomyopathy with an estimated prevalence of 1:200 in the general population and is caused by mutations in genes encoding sarcomeric proteins, the contractile machinery of cardiomyocytes. Over 1400 mutations have been identified to be causative of HCM, the majority of which residing in thick-filament genes (MYH7, MYBPC3) and to a lesser extent in thin-filament genes (TNNT2, TNNI3, TPM1, ACTC1, MYL2, MYL3). In recent years significant knowledge has been gained on the direct effects on sarcomere function of many of these mutations. However, exactly how altered sarcomere function ultimately gives rise to the HCM phenotype is a complex multifactorial process. Elucidation is warranted in order to identify and design novel therapeutic strategies tailored to different disease stages.

Currently it is hypothesized that sarcomere inefficiency, caused by mutant sarcomeric protein expression, perturbs cardiac energetics, forming the basis of the pathophysiology of HCM. Sarcomeres harboring mutant proteins are more sensitive to Ca2+, causing an increase in ATP consumption, and additionally require more ATP to generate tension compared to healthy

sarcomeres. High ATP demand and consumption elevate ADP levels both in the cytosol, which contributes to diastolic dysfunction through a Ca2+-sensitizing effect on the myofilaments, and in the mitochondria, which increases oxidation of NADH and NADPH, resulting in a disrupted NADH/NAD+ balance and a reduced capacity to detoxify ROS. Furthermore, as a consequence of increased Ca2+ binding at the myofilaments, less Ca2+ is available to regenerate NADH via the Krebs cycle. Together this represents an initial mechanism underlying mitochondrial and diastolic dysfunction, occurring early before onset of HCM. Subsequently a vicious cycle ensues of increasing mitochondrial and diastolic dysfunction, leading to impaired coronary perfusion and ischemia, which further exacerbates mitochondrial dysfunction and oxidative stress, ultimately leading to cardiac remodeling[1].

Four HCM mouse models (two MYBPC3 and two TNNT2 mutants) will be deployed at 1, 4 and 12 months of age to assess the processes underlying the hypothesized sequential changes in metabolism and mitochondrial function. In addition to mitochondrial respirometry, an array of techniques will be used to perform in vivo analyses of cardiac energetic status, perfusion and diastolic performance and in vitro analyses of cardiac substrate utilization, metabolites, proteins, contractile function and cell and tissue structure. The combined knowledge obtained from these studies will improve our understanding of the pathophysiology underlying HCM and identify therapeutic targets to be applied in (pre-)symptomatic individuals.

3. **K. Can, C. Menzfeld, P. Rehling, S. Kügler, J. Dudek, M. Müller (2018) Mitochondrial dysfunction in a mouse model of Rett syndrome.**

At the request of the author, this abstract is not made available online.

4. **Kumar A (2018) Ethanol impairs mitochondrial functions and ATP synthesis in skeletal muscle in alcoholic liver diseases**

At the request of the author, this abstract is not made available online.

5. **Kumar A (2018) L-Isoleucine reverses the hyperammonemia induced skeletal muscles mitochondrial dysfunction**

At the request of the author, this abstract is not made available online.

6. **Alexander Nickel, Edoardo Bertero, Michael Kohlhaas, Mathias Hohl, Carolin Krug, Andreas Müller, Michael Lafontaine, Roy Lancaster, Reinhard Kappl, Karina von der Malsburg, Martin van der Laan, Jan Dudek, Peter Rehling, Christoph Maack (2018) Defects in mitochondrial calcium uptake precede defects of the respiratory chain in X-linked Barth syndrome cardiomyopathy.**

At the request of the author, this abstract is not made available online.

7. **Pchelin P., Glyavina M., Loginov P., Shchelchkova N., Mukhina I. (2018) Activation of heterodimeric receptor to erythropoietin with its agonist CdEPO regulates brain mitochondrial bioenergetics after local acute ischemia/reperfusion in C57BL/6 mice. Mitochondr Physiol Network 23.06.**

Ischemic lesions remain to be one of the main causes of physical disability and mortality worldwide. Furthermore, stroke is known to be followed by mitochondrial dysfunction and impaired cell respiration. Mounting evidence demonstrates that the cytokine hormone erythropoietin (EPO) is capable of activating signaling pathways that increase the brain's resistance to ischemia/reperfusion stress. After the discovery of EPO's heteroreceptor that promotes tissue protection [1], a number of attempts were made to develop non-hematopoietic EPO's derivatives, including CdEPO. However, the precise mechanisms implicated into protective CdEPO effect, notably on brain mitochondria, are still to be elucidated.

The purpose of current research is to elucidate the effect of non-hematopoietic derivative of erythropoietin (CdEPO) on brain mitochondria respiration rate on 4, 10 and 20 day after local acute ischemia/reperfusion in mice.

Male C57BL/6 mice (2 months old, weighing 18-23 g) were used in the study. Local acute ischemia in mice was induced with transient middle cerebral artery occlusion (tMCAO). Following 6 hours after ischemic exposure a fivefold intravenous CdEPO administration was carried out. In a control group the administration of sodium chloride was performed in the same conditions. On 4, 10 and 20 day after reperfusion forebrains of animals were dissected to obtain isolated mitochondria. Bioenergetic studies were carried out using high-resolution respirometry (OROBOROS Oxygraph-2k). Significant difference (at least $p<0.05$) was tested by one-way ANOVA and Holm-Sidak post hoc.

It was revealed that ischemia/reperfusion with tMCAO did not lead to significant alterations in LEAK (glutamate and malate) respiration (Fig. 1 A). Intravenous CdEPO administration following 6 hours after ischemia/reperfusion did not exert any effect on LEAK respiration rate compared to control level. However, on day 20 after reperfusion brain mitochondrial OXPHOS respiration showed a significant decrease by 41% ($p=0.01$) in the control group compared to intact level (Fig. 1 B). Along with that on day 20 OXPHOS respiration rate was increased by 35% ($p=0.027$) in the CdEPO group in comparison with control level.

The observed effect of CdEPO on forebrain mitochondrial bioenergetics might be implicated in the realization of protective mechanisms, which was induced by EPO's heteroreceptor activation, and resulted in postponed improvement of mitochondrial respiration after ischemia/reperfusion. Other effects of CdEPO on different parameters of mitochondrial bioenergetics require further investigation.

MiPNet23.06 Abstracts IOC130: No presentation

Nicole MacDonald, Mahmoud Sharaf and Collins Kamunde (2018) H2O2 metabolism in liver and heart mitochondria: low emitting-high scavenging and high emitting-low scavenging systems. Mitochondr Physiol Network 23.06.

Although mitochondria are presumed to emit and consume reactive oxygen species (ROS), the quantitative interplay between the two processes in ROS regulation is not well understood. Here, we probed the role of mitochondrial bioenergetics in H2O2 metabolism using rainbow trout liver and heart mitochondria. Both liver and heart mitochondria emitted H2O2 at rates that depended on their metabolic state, with the emission rates (free radical leak) constituting 0.8 to 2.9% and 0.2 to 2.5% of the respiration rate in liver and heart mitochondria, respectively. When presented with exogenous H2O2, liver and heart mitochondria consumed it by first order reactions with half-lives (s) of 117 and 210, and rate constants of 5.96 and 3.37 (× 10-3 s-1), respectively. The mitochondrial bioenergetic status greatly affected the rate of H2O2 consumption in heart but not liver mitochondria. Moreover, the activities and contribution of H2O2 scavenging systems varied between liver and heart mitochondria. The significance of the scavenging systems ranked by the magnitude (%) of inhibition of H2O2 removal after correcting for emission were, liver (un-energized and energized): catalase > glutathione (GSH) ≥ thioredoxin reductase (TrxR); un-energized heart mitochondria: catalase > TrxR > GSH and energized heart mitochondria: GSH > TrxR > catalase. Notably, depletion of GSH evoked a massive surge in H2O2 emission that grossly masked the contribution of this pathway to H2O2 scavenging in heart mitochondria. Irrespective of the organ of their origin, mitochondria behaved as H2O2 regulators that emitted or consumed it depending on the ambient H2O2 concentration, mitochondrial bioenergetic state and activity of the scavenging enzyme systems. Indeed, manipulation of mitochondrial bioenergetics and H2O2 scavenging systems caused mitochondria to switch from being net consumers to net emitters of H2O2. Overall, our data suggest that the low levels of H2O2 typically present in cells would favor emission of this metabolite but the scavenging systems would prevent its accumulation.

Accommodation and location

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

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
>2,200 O2k-Publications – http://wiki.oroboros.at/index.php/O2k-Publications: Topics

COST Action CA15203 MitoEAGLE

MitoEAGLE preprint publication
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