Oroboros **O2k-Protocols**
mt-Preparations

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Tissue homogenates for diagnosis of mitochondrial respiratory function: mouse heart, brain and liver

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1. Introduction

A high-quality preparation of tissue homogenate may represent an optimum compromise for a variety of respirometric and fluorometric studies. These considerations provided the rationale for initiating a study with the PBI-Shredder, an auxiliary HRR-Tool providing a standardized approach to prepare homogenates of various tissues (e.g. heart, liver, brain) with high reproducibility of mitochondrial yield and mitochondrial function as evaluated with HRR.

2. Sample preparation

2.1. Organ harvest

Heart, liver and brain are excised from the sacrificed mouse and added into Falcon tubes containing sufficient ice-cold BIOPS (mouse heart) or respiration medium (mouse liver and brain) to cover the entire tissue sample. Keep on ice and minimize transportation and storage time as far as possible.

2.2. Tissue preparation and homogenization (shredding)

Prepare tissue samples of about 4 mg wet weight (W_w) for mouse heart muscle and 8 mg W_w for mouse brain and liver for two O2k-Chambers (half the W_w if one Shredder Tube should be used for one O2k-Chamber).

Preparation of tissue homogenate and determination of the wet weight (W_w) has to be done according to MiPNet17.02.

3. Experimental setup with the Oroboros O2k

Respiration of all tissue homogenates was measured in respiration medium at 37 °C and in a normoxygen range. For detailed describtion see MiPNet17.02.

4. Experimental protocol

Substrate-uncoupler-inhibitor-titration (SUIT) protocols were used to evaluate the mitochondrial function of mouse heart, mouse brain and mouse liver homogenate.

All substrates, inhibitors and the uncoupler used in the protocols with mouse heart, brain and liver homogenate can be found in MiPNet09.12.

5. Results

In the present methodological evaluation of tissue homogenates prepared with the PBI-Shredder SG3, results are expressed per mg of tissue applied for preparation per O2k-chamber (2 ml). Representative results of two chambers are presented for each tissue homogenate.

6. Mouse heart homogenate

6.1. High-Resolution FluoRespirometry (HRFR)

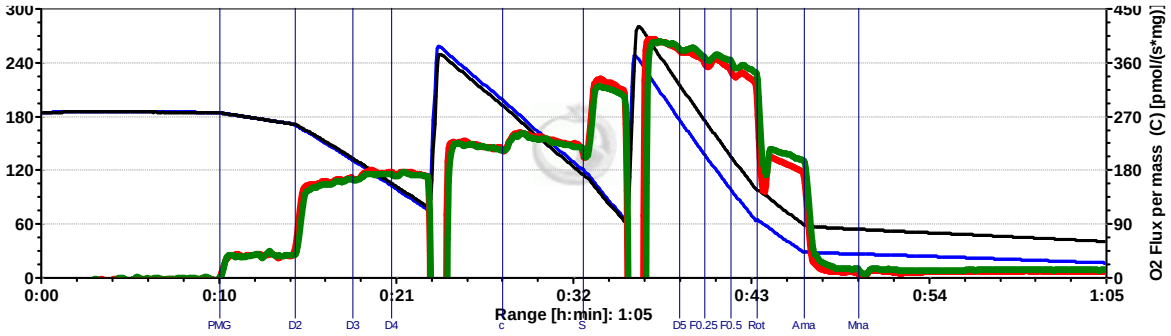

Fig. 1: Overlay of O2k-traces of two chambers. Oxygen concentration [µM] (black and blue line) and oxygen flux per mass [pmol·s^{-1}·mg^{-1}] (red and green line) of mouse myocardium in MiR05Cr.

6.2. O_2 flux per tissue mass [pmol·s^{-1}·mg^{-1}] and *FCR*

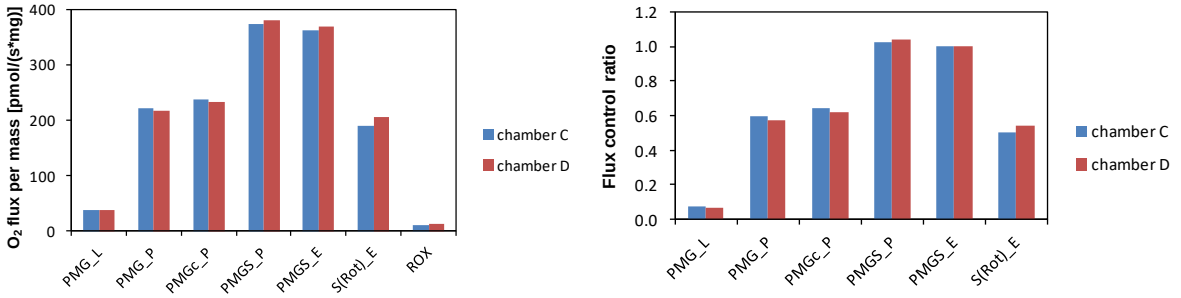

Fig. 2: O_2 flux per mass [pmol·s^{1}·mg^{-1}] and flux control ratio normalized to $PMGS_E$ and corrected for ROX, of chamber C and chamber D, in mouse heart homogenate.

6.3. Coupling control ratios and substrate control ratios

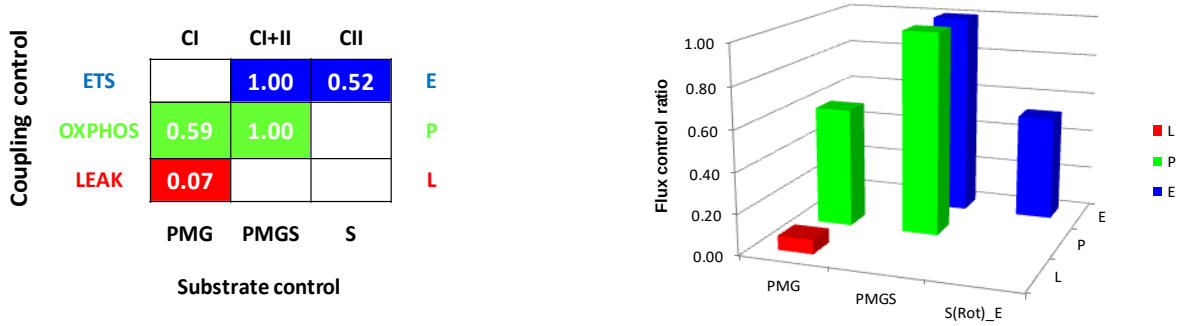

Fig. 3: Coupling control and substrate control ratios for mouse heart homogenate, calculated as mean of chamber C and chamber D.

7. Mouse brain (cortex) homogenate

7.1. High-Resolution FluoRespirometry (HRFR)

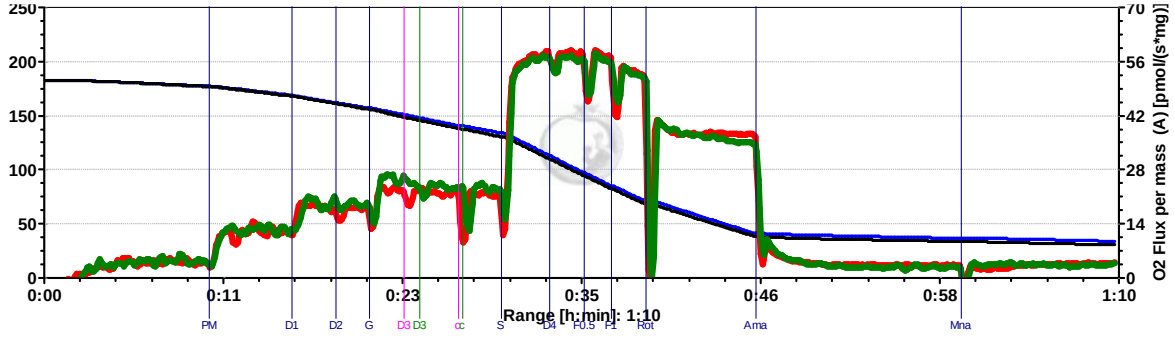

Fig. 4: Overlay of O2k-trace of chamber A and chamber B. Oxygen concentration [µM] (black and blue line) and oxygen flux per mass [pmol·s^{-1}·mg^{-1}] (red and green line) of mouse brain (cortex) homogenate in MiR05.

7.2. O_2 flux per tissue mass [pmol·s^{-1}·mg^{-1}] and *FCR*

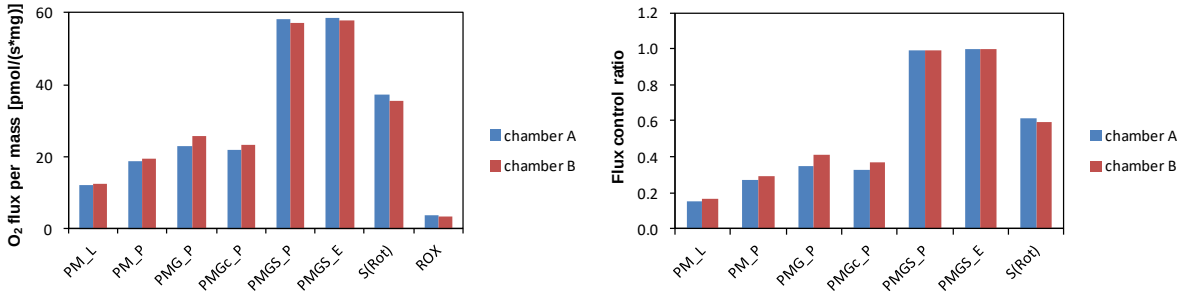

Fig. 5: O_2 flux per mass [pmol·s^{-1}·mg^{-1}] and flux control ratio normalized to $PMGS_E$ and corrected for ROX, of chamber A and chamber B, in mouse brain (cortex) homogenate.

7.3. Coupling control ratios and substrate control ratios

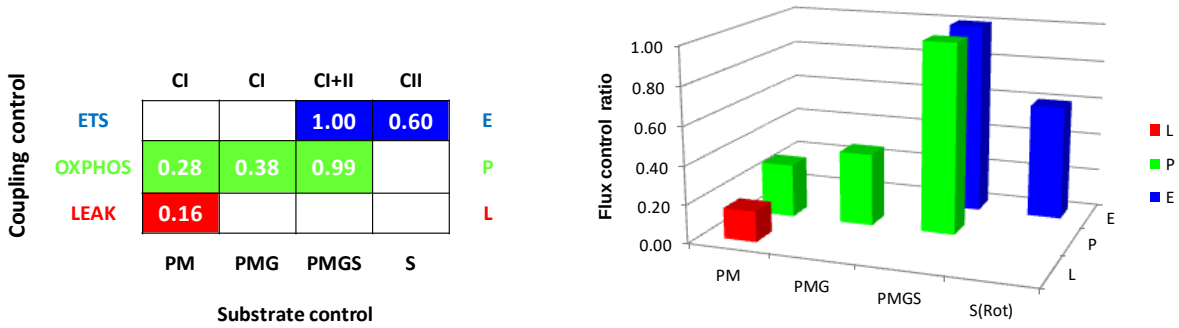

Fig. 6: Coupling control and substrate control ratios for mouse brain (cortex) homogenate, calculated as mean of chamber A and chamber B.

8. Mouse liver homogenate

8.1. O2k High-Resolution FluoRespirometry (HRFR)

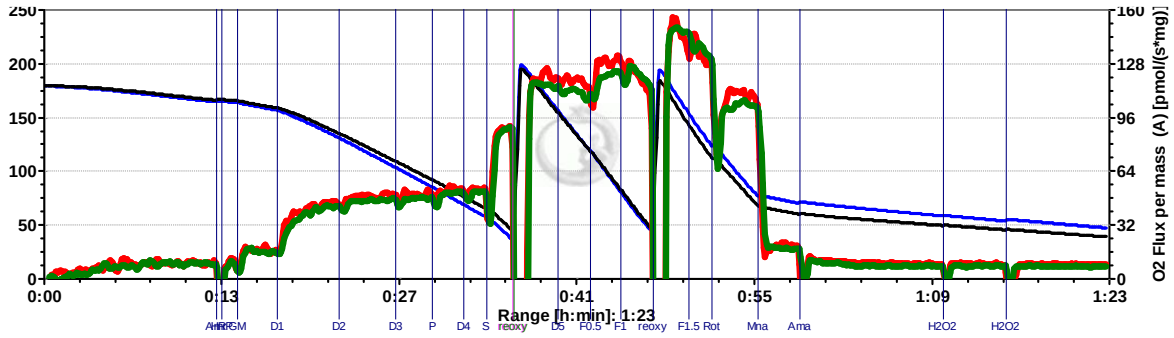

Fig. 7: Overlay of O2k-trace of chamber A and chamber B. Oxygen concentration [µM] (black and blue line) and oxygen flux per mass [pmol·s^{-1}·mg^{-1}] (red and green line) of mouse liver homogenate in MiR05.

8.2. O_2 flux per tissue mass [pmol·s^{-1}·mg^{-1}] and FCR

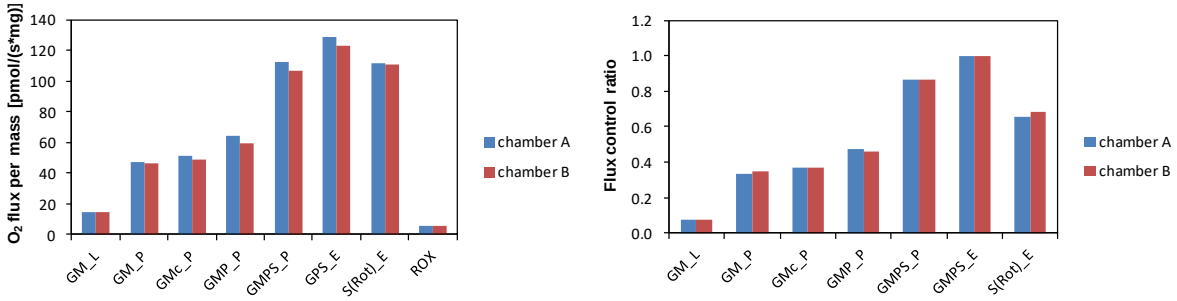

Fig. 8: O_2 flux per mass [pmol·s^{-1}·mg^{-1}] and flux control ratio normalized to $PMGS_E$ and corrected for ROX, of chamber A and chamber B, in mouse liver homogenate.

8.3. Coupling control ratios and substrate control ratios

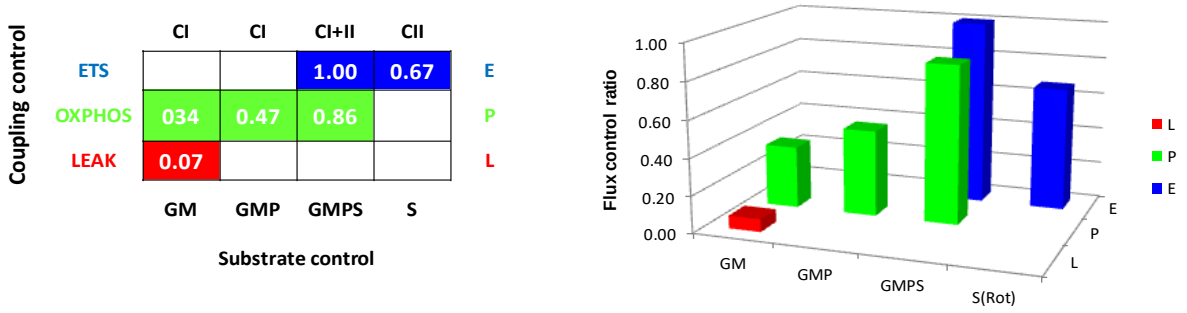

Fig. 9: Coupling control and substrate control ratios for mouse liver homogenate, calculated as mean of chamber A and chamber B.

9. Conclusions

The application of an additional tool to remove the serrated Shredder Ram as well as the Shredder Cap after homogenization increased the mitochondrial yield by washing out the homogenate completely from both sides of the Lysis Disk.

The cytochrome *c* effect could be diminished in mouse heart homogenate and no cytochrome *c* effect occurred in mouse liver and mouse brain homogenate.

10. Acknowledgements

Contribution to K-Regio project *MitoCom Tyrol*, funded in part by the Tyrolian Government and the European Regional Development Fund (ERDF).
www.bioblast.at/index.php/K-Regio_MitoCom_Tyrol

11. References

Mitochondr Physiol Network – MiPNet Manuals and Protocols

MiPNet09.12: Oroboros O2k titrations. Mitochondria, permeabilized cells and biopsies. Mitochondr Physiol Network 09.12.
MiPNet17.02: PBI-Shredder HRR-Set: Preparation of tissue homogenates for diagnosis of mitochondrial respiratory function.

12. Author contributions and publication versions

Eigentler A performed experiments and wrote the manuscripts. Fontana-Ayoub M performed experiments. Gnaiger E designed experiments and edited the manuscript.

Results for fish (trout) homogenate of liver and heart are published in MiPNet17.03: Doerrier C, Draxl A, Wiethuechter A, Eigentler A, Gnaiger E. Mitochondrial respiration in permeabilized fibres versus homogenate from fish myocardium and liver. An application study with the PBI-Shredder. Mitochondr Physiol Network 17.03.

Preliminary results for mouse heart homogenate versus permeabilized fibres can be found in the appendix of MiPNet17.03.