Mitochondrial respiration in different cell lines measured in the 2.0 mL and 0.5 mL chambers of the O2k-FluoRespirometer (Oroboros): an experimental basis for platform comparison with the Seahorse XFe24 Bioanalyzer (Agilent)

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This project is focused on measuring mitochondrial respiration on permeabilized fibroblast cell lines from patients with congenital disorders of glycosylation, because our preliminary results indicate secondary functional abnormalities in mitochondria and glycolytic dysfunction due to a breakdown of the glycosylation pathway. The growth of fibroblast cell lines from patients with congenital disorders of glycosylation is the most limiting factor for our measurements to this date and smaller amount of cells would help us to get more data in shorter time. According to this fact, I participated on optimizing new method using smaller chambers on O2k-FluoRespirometer during STSM. This method with modified type of SUIT1 and SUIT2 protocols and smaller chambers would really help to our project, on which I am focused in my postgraduation studies. Furthermore STSM will be very helpful for me to achieve a lot of new experiences with measuring on O2k-FluoRespirometer in most specialized laboratory in the world for mitochondrial respiration measurements on this instrument and will be used a lot in our following-up project.

These results could help also to other scientists working with cell cultures and measuring mitochondrial respiration in O2k-FluoRespirometer to get their results in shorter time because of reduced material requirements. Especially scientists using fibroblast cell lines from patients with mitochondrial disorders and with congenital disorders of glycosylation or other diseases could appreciate this reduced amount. Thus, STSM will be focused to target specific goals in MoU i.e. “Providing standardized measurements to link mitochondrial and physiological performance to understand the myriad of factors that play a role in mitochondrial physiology” and devoted to complete specific WG1 and WG4.

First part of this STSM was focused on comparing measurement results of the 0.5 and 2.0 mL chambers with HEK293 cells and thereafter with control fibroblast cell lines by using optimized SUIT protocols. Results of STSM will serve as background for additional experiments on Seahorse XFe24 Bioanalyzer and O2k-Respirometer provided in our laboratory in Prague. The identical SUIT-3 protocol and identical conditions will be used for quantitative comparison of results obtained by the Seahorse XFe24 Bioanalyzer with attached fibroblasts, and by the O2k-FluoRespirometer with suspended cells. The output of this study is planned to be finally published.