COMPARTMENT-SPECIFIC METABOLOME ANALYSIS REVEALS THE TIGHT LINK BETWEEN IgG1 FORMATION AND NECESSARILY HIGH MITOCHONDRIAL SHUTTLE ACTIVITIES IN CHINESE HAMSTER OVARY CELLS

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Chinese hamster ovary (CHO) cells are the dominating host for the production of pharmaceutical proteins, in particular monoclonal antibodies (mABs). Although production titers improved more than 100 fold during the last 2 decades, similar enhancements of cell specific productivities are less pronounced. They demand for detailed subcellular studies to identify promising metabolic engineering targets. In this context, our study focused on compartment specific metabolome analysis to measure metabolic patterns in the cytosol and in the mitochondrion during cell cultivation. Thereof, in vivo shuttle activities were calculated and correlated with cell specific IgG1 formation rates.

The compartment-specific metabolome and labelling analysis (13C) distinguishes between cytosol and mitochondrion. Metabolomics and instationary 13C metabolic flux analysis build on preliminary own studies of 13C analytics (Teleki et al., *Anal Biochem* 2015; Teleki et al. *Metab Eng* 2017) and compartment-specific metabolomics (Matuszczyk et al., *Biotechnol J* 2015; Pfitzenmaier et al., *Biotechnol J* 2016). Further development and optimization has been performed finally reaching the current status that allows monitoring compartment-specific flux distributions and shuttle activities during the course of cell cultivation.

Studying multiple periods of an IgG1 production process the crucial role of the mitochondrion not only as a provider of ATP but also as an essential part of metabolism was unraveled. 13C flux analysis disclosed the time-variant activities of the mitochondrial shuttles that are tightly linked to mitochondrial and cytosolic metabolism. Clear evidence was found that mAB production strongly depends on sufficient NADPH supply provided by cytosolic malic enzyme activity and malate export from the mitochondrion.