INVESTIGATION OF NADPH OXIDASE ACTIVATION IN (NON-INNATE IMMUNE) CELLS USING NAD(P)H-FLIM

Daniel Bremer, Ronja Mothes, Agata Mossakowski, Helena Radbruch, Raluca Niesner
German Rheumatism Research Center (DRFZ)
Charitéplatz 1, D-10117 Berlin, Germany
E-mail: daniel.bremer@drfz.de

KEY WORDS: FLIM, NADPH oxidase, reactive oxygen species, multiple sclerosis, living cells.

FLIM has proven to be a reliable, quantitative tool to probe vital processes and metabolic functions in living cells. NADPH oxidases (NOX) are membrane-bound enzyme complexes that catalyse the reduction of free oxygen to its superoxide anion, which further leads to reactive oxygen species (ROS) production. Oxidative stress caused by massive ROS production (mainly catalyzed by NOX2) is thought to be a major factor in different pathologies, e.g. promoting neuronal damage in multiple sclerosis (MS).

In our previous work we employed NAD(P)H (i.e. NADH and NADPH) fluorescence lifetime imaging to identify functional NOX in isolated murine and human immune cells as well as in living mice. We demonstrated that the increase of fluorescence lifetime of NAD(P)H is specific for binding NADPH to members of the NOX family in murine polymorphonuclear cells [1]. We also showed that in vivo fluorescence lifetime imaging reveals NADPH oxidase overactivation in monocytes, microglia and astrocytes as a main mechanism of multiple sclerosis pathogenesis [2]. How the metabolic activation in cell others than innate immune cells, e.g. B cells, T cells, neurons, etc. changes during chronic inflammation remains elusive and is subject of this study.

Figure 1: Lifetime map of cellular metabolism in living B cells from healthy C57BL/6 mouse genotype. Left: Unstimulated B cells. Right: Stimulated B cells.
