University of Copenhagen

Master Thesis Project

Cellular mechanism of action studies of novel dimeric p47phox inhibitors

PROJECT BACKGROUND

NOX2 and Oxidative Stress: Reactive oxygen species (ROS) produced through the expression of NADPH Oxidase (NOX2; Figure 1, left) are normally counteracted by antioxidants to maintain tissue redox homeostasis. However, in chronic inflammatory conditions, surplus production of oxidants by macrophages directly destroy tissue components and disrupt cellular redox signaling. A multitude of genetic and pharmacological studies have suggested that inhibition of NOX2 is beneficial in relation to several diseases (e.g. stroke, neurodegeneration, rheumatoid arthritis, acute lung inflammation). For decades it has been attempted to correct the imbalance between oxidants and antioxidants by the development of NOX2 inhibitors, but all trials have failed. With this project we introduce a conceptually novel way of inhibiting NOX2 by interfering with the recruitment of cytosolic cofactors (p47phox; Figure 1, right) instead of inhibiting the core enzymatic portion of NOX2 (flavocytochrome b_{568}).

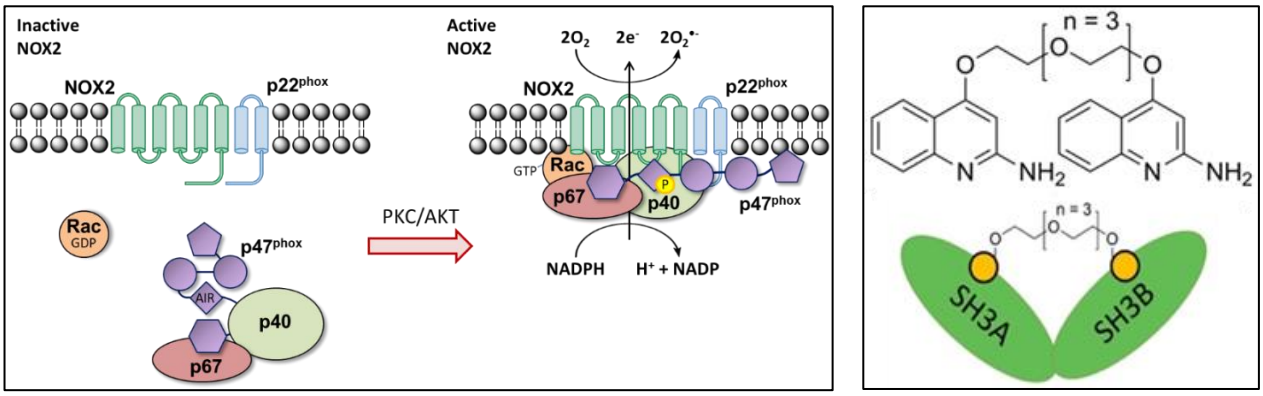

Figure 1. *Left:* In the inactive state the various subunits of the NOX2 complex are found in their resting conformations. Phosphorylation of the regulatory subunit p47phox (by e.g. PKC) induces conformational changes that relieve autoinhibition by the SH3A and SH3B domains, and p47phox assembles with the NOX2 core enzyme in the membrane to commence superoxide production. *Right:* We have developed a novel type of NOX2 inhibitors that bind the tandem SH3A-B domain of p47phox and thereby prevent NOX2 activation.

Dimeric NOX2/p47phox inhibitors: By fragment-based drug discovery (FBDD) we have developed novel protein-protein interaction inhibitors of the p47phox subunit of NOX2 (Solbak *et al.*, J. Med. Chem. 2020, 1156–1177), with good affinities towards p47phox (K_i ~0.4 µM), and a promising effect in macrophage assays for NOX2 activation and superoxide production.

AIM OF PROJECT

With this master thesis project, we wish to further characterize the cell activity of the compounds using various outputs for NOX2 activity and cellular target engagement. Thereby, we hope to gain understanding of their exact mechanism of action in relevant cell systems. You will learn to do typical techniques within in vitro pharmacology and cellular biology – e.g. cell culturing, transfections, redox activity assays, fluorescence microscopy, and Western blotting – and gain insight into redox biology, which is a highly relevant area of drug discovery and pharmacology.

- The project is carried out in collaboration between Assoc. Prof. Anders Bach (Dept. Drug Design and Pharmacology) and Assoc. Prof. Frederik Vilhardt (Dept. Cellular and Molecular Medicine). The lab work will be performed in Frederik's lab at Panum and you will become an integrated member of both research groups.

- For more information, please send CV and motivations to anders.bach@sund.ku.dk and/or vilhardt@sund.ku.dk