Grenoble Alliance for Integrated Structural & Cell Biology

GRAL MSc RESEARCH SCHOLARSHIP 2020-2021

RESEARCH INTERNSHIP PROPOSAL

Institute / Group

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Research Project Title

Engineering production of NADPH oxidase NOX2/p22^{phox} using lentiviral expression system: Towards structural characterization and protein therapy of X-linked chronic granulomatous disease (X-CGD).

Description of the project

The aim for the M2 internship is to set up a production strategy of the heterodimer NOX2/p22^{phox} by lentiviral transduction in the human mammalian cells HEK293T using an efficient protocol for expressing membrane proteins (Elegheert Nature Protocols 2018). After development of a purification procedure, the recombinant NOX2/p22^{phox} will be reconstituted into liposomes and its NADPH oxidase activity will be tested in a cell-free-system assay [Brault et al. Int J Nanomedicine. (2017)]. Once developed, this process will open avenues to future protein therapy for X-CGD and also structural characterization of this membrane protein alone or in its activated state (complexed with its cytosolic partners p67^{phox}, p47^{phox} and Rac) using neutron scattering and cryo-electron microscopy.

Experimental techniques: NOX2 and p22^{phox} will be cloned in transfer plasmids and co-transfected in HEK293T Lenti-X cells with envelope and packaging plasmids to generate viral particules. Expression analysis of NOX2/p22^{phox} will be followed by western-blot and flow cytometry (techniques available in the lab). NADPH oxidase activity of NOX2/p22^{phox} in liposomes will be controlled in a cell free system assay in presence of recombinant cytosolic partners p47^{phox}, p67^{phox} and Rac proteins routinely produced in the lab.

Recommended background: Cell culture, biochemistry, cloning, recombinant expression, flow cytometry, activity test.

Keywords

Recombinant NOX2/p22^{phox}, X linked CGD, lentiviral transduction, proteoliposome therapy, structural analysis of macromolecular membrane complexes

Relevant publications of the team

Beaumel S, Stasia MJ. The X-CGD PLB-985 Cell Model for NOX2 Structure-Function Analysis. Methods Mol Biol. 2019; 1982:153-171.

Brault J, Vaganay G, Le Roy A, Lenormand JL, Cortes S, Stasia MJ. Therapeutic effects of proteoliposomes on X-linked chronic granulomatous disease: proof of concept using macrophages differentiated from patient-specific induced pluripotent stem cells. Int J Nanomedicine. 2017; 12:2161-2177.

Hajjar C, Cherrier MV, Dias Mirandela G, Petit-Hartlein I, Stasia MJ, Fontecilla-Camps JC, Fieschi F, Dupuy J. The NOX Family of Proteins Is Also Present in Bacteria. MBio. 2017 ;8(6). pii: e01487-17