Host-pathogen interactions in Plants: Characterization of NLPs, a new family of pore-forming proteins

Necrosis- and ethylene-inducing 1-like proteins (NLPs) are important virulence factors of plant-associated microorganisms such as bacteria, fungi and oomycetes (*1, 2*). NLPs were shown to exhibit cytotoxicity towards plant cells and hence promote infections and toxic effects. NLPs damage plant lipid membranes through a multi-step mechanism that involves binding to plant sphingolipids, glycosyl inositol phosphoceramides (GIPC) (*3*), oligomerisation at the plant membrane level and, finally, disrupting the lipid membrane by forming small, transient pores. Membrane damage by pore formation used by NLPs is different from other families of pore forming toxins and is adapted to plant target membranes (*4*). Although basic steps of membrane-damage mechanism of NLPs are known, crucial details on understanding on molecular assemblies formed at the surface of lipid membrane are still missing. We are investigating this process using structural biology, biophysical and biochemical approaches to gain unique insights into NLPs membrane damaging mechanism. The aim of the project will be to characterize NLPs pore with structural biology approaches. Understanding molecular damage induced by NLPs at the molecular level will enable the development of strategies to inhibit NLP activity and improve crop's health.

The doctoral fellow will be trained in protein production and purification as well as in biochemical and biophysical approaches to study molecular assemblies of NLPs formed at the surface of lipid membranes. The doctoral fellow will prepare recombinant NLPs and use model membranes, such as liposomes and lipid nanodiscs, to image any oligomers composed of NLPs by cryo-electron microscopy.

The National Institute of Chemistry is fully equipped with state of the art instruments for carrying out the research project (https://www.ki.si/en/about-the-institute/research-infrastructure/), including a Glacios 200 kV cryoTEM. The Institute is part of the MOSBRI project, where the doctoral fellow can perform secondments, whenever other complementary techniques be needed to complete the characterization of the NLP pores.

References:

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2. K. Pirc, I. Albert, T. Nurnberger, G. Anderluh, Disruption of plant plasma membrane by Nep1-like proteins in pathogen-plant interactions. *The New phytologist*, (2022).
3. T. Lenarcic *et al.*, Eudicot plant-specific sphingolipids determine host selectivity of microbial NLP cytolysins. *Science* **358**, 1431-1434 (2017).
4. K. Pirc *et al.*, An oomycete NLP cytolysin forms transient small pores in lipid membranes. *Science Advances* **8**, eabj9406 (2022).

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Proposed collaboration within ArchiFun network:

Main ArchiFun theme involved:

x Host-pathogen interactions;

Mechanisms of bacterial resistance and cancer onsets;

Neurodegenerative and autoimmune diseases;

Translational research in prevalent diseases;

Physiology and ecology;

Neurosciences and cognition.