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Structure and Assembly Mechanism of the Ninjurin-1 Membrane Perforation Pore in Executing Cell Death

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Summary. The rupture of the eukaryotic plasma membrane mediated by the membrane protein ninjurin-1 is the defining end point of multiple different types of cell death. We aim at deciphering the mechanisms of ninjurin-1 in assembling membrane rupturing pores by a unique combination of structural biology methods, nanotechnological approaches, and super-resolution light microscopy.

Background. Eukaryotic cells possess remarkable control mechanisms to retain functionality during their life cycle and to ensure their proper removal in the multicellular context.[1,2] Among these mechanisms are multiple forms of cell death, most of which end with the rupture of the plasma cell membrane. So far, rupture of this membrane was generally thought to be a consequence of osmotic pressure, caused by uncontrolled influx of water. It was recently discovered that plasma membrane rupture is an active process, mediated by the protein ninjurin-1.[3] Ninjurin-1 is a ubiquitously expressed, 16-kDa plasma membrane protein, and conserved from fruit fly to man.[4] Understanding this process at atomic level will be crucial for therapeutic intervention.

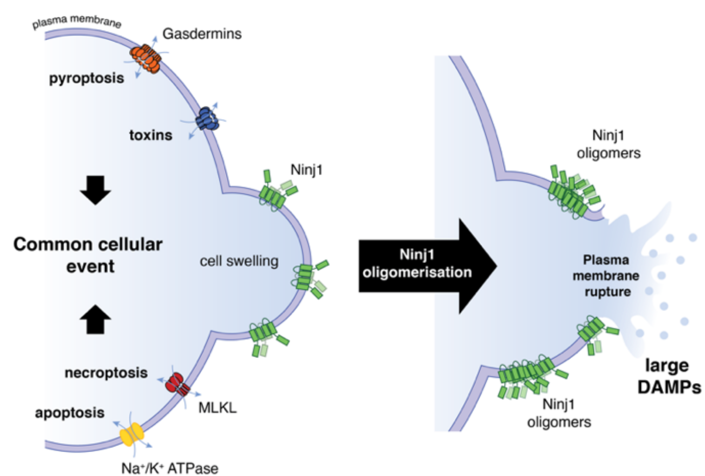

Figure 1. Ninjurin-1-mediated plasma membrane rupture as an endpoint to different cell death pathways. Execution of pyroptosis and necroptosis involves the formation of gasdermin pores, MLKL channels, pore-forming toxins, or the Na^+/K^+ ATPase. The resulting membrane bubbles rupture upon oligomerization of ninjurin-1 and release cytosolic content that acts as damage-associated molecular patterns (DAMPs).

Goal of this thesis. We want to determine the structure of the ninjurin-1 membrane inserted pore at atomic level and unravel its insertion mechanism in the cellular context. We aim at the atomic resolution structure of the ninjurin-1 pores by single-particle cryo-electron microscopy.[5,6] In addition, we will characterize the ninjurin-1 system by NMR spectroscopy.[6] In parallel, we will monitor ninjurin-1 oligomeric assembly and pore formation by AFM and TEM.[7–10] Time-lapse multiparametric AFM will allow us to image the pore formation process at a resolution of ≈1-2 nm and to simultaneously map how ninjurin-1 insertion and assembly alters the mechanical properties of the target membrane. Along a third line of research, we will also study the pore assembly in living cells by MinFlux microscopy, which allows an isotropic resolution of 1-5 nm of fluorophores in 3D.[11,12]

Collaboration between the labs. This thesis follows a long standing and highly fruitful collaboration between the Hiller and Müller labs, combining their complementary expertise in biophysics and structural biology.[7,10,13] With the relocation of the D-BSSE to the Schellenmätteli campus in mid 2022, the two labs will be next neighbors. The student will be embedded in both groups and have full infrastructure access at both departments.

References. (1.) Broz P et al. Nat Rev Immunol 20, 143–157 (2020). (2.) Broz P et al. Nat Rev Immunol 16, 407–420 (2016). (3.) Kayagaki N et al. Nature 591, 131–136 (2021). (4.) Araki T et al. Neuron 17, 353–361 (1996). (5.) Kaur H et al. Nature 593, 125–129 (2021). (6.) Sborgi L et al. Proc Natl Acad Sci USA 112, 13237–13242 (2015). (7.) Sborgi L et al. EMBO J 35, 1766–1778 (2016) (8.) Dufrêne Y et al. Nat Methods 10, 847–854 (2013). (9.) Dufrêne Y et al. Nat Nanotech 12, 295–307 (2017). (10.) Mulvihill E et al. EMBO J 37, e98321 (2018). (11.) Gwosch KC et al. Nat Methods 17, 217–224 (2020). (12.) Schmidt R et al. Nat Commun 12, 1478 (2021). (13.) Thoma J et al. Nat Struct Mol Biol 22, 795–802 (2015).