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Engineering listeriolysin O for specific pore formation *in vitro*

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Listeriolysin O (LLO) is the pore forming toxin and the most important virulence factor of bacteria *Listeria monocytogenes*, which causes food-borne disease listeriosis. It belongs to cholesterol-dependent cytolysins (CDC), a family of pore-forming toxins produced primarily in Gram positive bacteria. LLO enables bacteria to escape from host phagolysosome and to spread into the neighboring cells. Moreover, LLO is a unique protein among CDC, since its stability is pH dependent. Pore forming proteins, such as α -hemolysin, have shown their way to biotechnological applications (e.g., DNA sequencing through nanopore). LLO also found its potential use in medicine as a vaccine adjuvant and carrier molecule for anti-tumor therapies and gene delivery. We believe that by revealing the exact mechanism of LLO pore formation, we can engineer LLO to form pores, which can be manipulated and used in biotechnological applications. First, we used giant unilamellar vesicles (GUVs) to study LLO's mechanism of pore formation. Confocal fluorescence microscopy and flow cytometry were used to analyze time, concentration and environment dependent pore formation of LLO. We discovered the damaging effect of LLO's pore formation on membranes by analyzing the permeability of GUVs for fluorescent dextrans (FDs) of different sizes and populations of GUVs upon LLO addition. LLO pore forming activity enabled time dependent permeabilization of FDs up to 150 kDa, which is larger than previously assumed pore diameter. LLO was able to damage the vesicles in such extend, that it led to their destruction. Furthermore, we used cell free *in vitro* synthetic biology approach to construct and produce LLO inside synthetic phospholipid vesicles, which can be specifically controlled by environmental changes.

Biography

Sasa Rezelj has completed her MSc from Biotechnical Faculty of the University of Ljubljana. She is the currently a PhD student in Department for Molecular Biology and Nanobiotechnology, National Institute of Chemistry, Slovenia. Her area of interest is synthetic biology approach to study and engineer listeriolysin O under supervision of Professor Dr. Gregor Andreluh.

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