

THE SPECIFICITY OF THE STREPTOCOCCUS PNEUMONIAE TOXIN PNEUMOLYSIN FOR LIPID BILAYER COMPOSITION

Adelė Unikauskaitė¹, Tadas Ragliauskas¹

¹Institute of Biochemistry, Life Sciences Center, Vilnius University
adele.unikauskaite@gmc.stud.vu.lt

The cell membrane is a crucial barrier, protecting cellular contents from the external environment while maintaining interactions with surrounding molecules. However, bacterial toxins, such as pneumolysin (PLY) [1], a cholesterol-dependent cytolysin from *Streptococcus pneumoniae*, can bind to membranes, form pores, and disrupt cellular homeostasis. The main objective is to investigate how variations in lipid bilayer composition influence PLY binding and pore formation, providing insights into the toxin's membrane selectivity.

Cholesterol is the major contributor to promoting PLY binding to lipid membranes and induction of ion-defect formation. However, the levels of cholesterol needed may vary on surrounding lipids. Also, the size and charge of the lipid headgroup interplay with the protein's access to cholesterol within the membrane; potentially, other physical properties of the lipid bilayer are affected. In this work, we use electrochemical impedance spectroscopy (EIS) to analyze the electrical properties of membranes, such as electric conductivity and capacitance. We use tethered lipid bilayers (tBLMs) [2] composed of unsaturated lipids with various headgroups (choline, ethanolamine, glycerol) and cholesterol. Permeability is determined by the minimum frequency, which reflects membrane resistance and capacitance changes, indicating protein binding and further pore formation.

The primary findings indicate that in two-lipid systems (cholesterol and zwitterionic lipid), the threshold of detection of pneumolysin activity requires at least 40 mol% of cholesterol. In more complex systems with the addition of other zwitterionic lipids, which affect bilayer packing, pneumolysin induces similar damage to the membranes but with lower cholesterol levels.

[1] Rubins and Janoff, 1998. Pneumolysin: a multifunctional pneumococcal virulence factor. *Journal of Laboratory and Clinical Medicine*, vol. 131, iss. 1, p. 21-27.

[2] Ragaliauskas et al. 2017. Fast formation of low-defect-density tethered lipid bilayers by fusion of multilamellar vesicles. *BBA Biomembranes*, vol. 1859, iss. 5, p. 669-678.