Towards Understanding the Mode-of action of Antimicrobial Peptides: what can we learn from model membranes and scattering techniques?

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It is generally believed that antimicrobial peptides, AMPs, are able to evade much of the bacterial resistance because they disturb the fundamental integrity of the entire cell by interfering with the life-defining cell membrane. However, there is no clear general consensus for the molecular basis by which AMPs act, although various structural modifications such as membrane deformation or pore formation have been suggested. [1,2]

In this project we systematically aim to understand the effect of AMP on lipid membranes focusing on both the structure and dynamics. By taking a representative selection of natural AMPs we systemically investigate structural effects on the lipid membrane, the mode of peptide insertion and specifically, whether transmembrane "pores" are formed. To this end we use small-angle X-ray and neutron scattering (SAXS/SANS) in combination with quantitative modelling and auxiliary techniques such as differential scanning calorimetry (DSC) and atomic force microscopy (AFM).[3-5] We furthermore investigated a series of AMPs that self-assemble into well-defined nanostructures and ask the question whether they are active as monomers or as assembled unit.

To gain insight into potential impact on the AMPs on the lipid membrane beyond structural pores, we investigate the dynamics, more specifically flip-flop and intermembrane diffusion processes of lipids using time-resolved SANS and a H/D contrast variation scheme. The results further show that although the structure of the peptide differs, all membrane active AMPs cause a markedly faster lipid dynamics. [5,6] By using various lipid mixtures and partial H/Dl labelling we show that this effect extend to various lipids (PC, PG, PE)[6,7]. Interestingly, by analysing the temperature dependent rate constants, we show that the acceleration of flip-flop dynamics is not necessarily of enthalpic origin but rather entropic. We will discuss the mechanism in light of recent computer simulation techniques

[1] H. Jenssen, P. Hamill and R. E. W. Hancock, Clinical Microbiology Reviews, **2006**, 19, 491–511.

[2] W. C., Wimley, *ACS Chemical biology* **2010,** *5* (10), 905-917.

[3] J.E. Nielsen, J. E., V:A. Bjørnestad, & R. Lund, Soft Matter, **2018**, 11, 37–14.

[4] Nielsen, J. E., Lind, T. K., Lone, A., Gerelli, Y., Hansen, P. R., Jenssen, H. M, Cárdenas and R. Lund *BBA - Biomembranes*, *1861*(7), 1355–1364.

[5] Nielsen, J. E.; Bjørnestad, V. A.; Pipich, V.; Jenssen, H.; Lund, R. *J. Coll. Int. Sci.* **2021**, *582*, 793–802.

[6] Nielsen, J. E.; Prevost, S.; Lund, R. *Faraday Discuss.,* **2021**, 232, 203.

[7] Nielsen, J. E. and Lund, R. *Langmuir,* **2022**, 38, 374−384.