Peptide-induced pore formation in lipid bilayers studied by fluorescence microscopy and computer simulations

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Many amphiphilic peptides have the ability to form transmembrane pores in lipid bilayers above certain threshold of peptide-to-lipid ratios. Such phenomenon occurs in a number of biological processes such as: lysis of membranes induced by antimicrobial peptides (AMPs), membrane transport, pore formation in mitochondria during apoptosis or membrane fusion occurring during the entry of enveloped viruses (Almeida and Pokorny, 2009; Fischer et al., 2005; Chernomordik and Kozlov, 2008). Several models of peptide-lipid interactions have been proposed. One usually mentions the **barrel-stave model** in which a pore is formed in the membrane without significant perturbation of the surrounding lipids and the **carpet/torroidal model** in which the peptides accumulate around the surface of the lipid membrane (Fischer et al., 2005) (Fig. 1). Regarding the kinetics of peptide-induced efflux (e. g. of the cellular content during lysis), one applies the so-called all-or-none and graded models (Almeida and Pokorny, 2009). In spite of a tremendous interest in membrane-interacting peptides in recent years, the details of their mode of action are not fully understood.

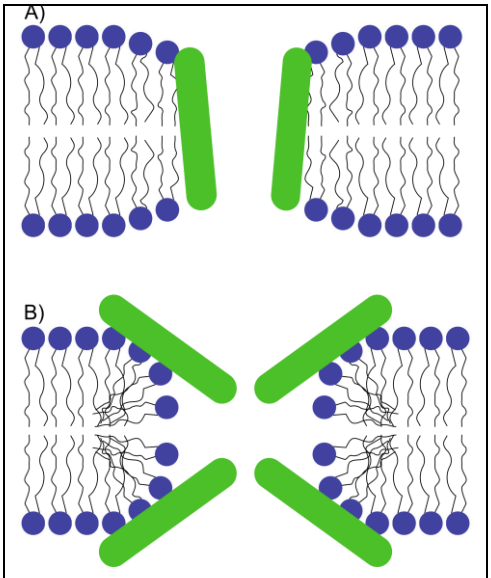

Fig. 1 Scheme of geometries in a peptide-induced pore in lipid bilayer according to A) a barrel-stave and B) carpet-torroidal models.

In this project, we would like to study the effects on lipid membranes induced by model amphiphilic peptides, with a special interest in the influenza virus fusion peptide derivatives. This peptide is the N-terminal fragment of hemagglutinin subunit HA2, anchoring in the internal membrane of cellular endosome, leading to its fusion with the viral membrane envelope during the viral entry. The last years of studies showed that adding three more residues to the well-studied 20-aminoacid fragment lead to a novel structure of a tight helical hairpin (Lorieau et al., 2010). Such structure can be also assigned from a hydrophobic moment map (Worch 2013). Molecular simulations showed that 20-aminoacid fragments are able to stabilize a highly fusogenic pre-fusion structure composed of a peptide bundle (Risselada et al., 2012). However, it is not known what are the effects of increasing the peptide length on such structure formation and/or peptide-to-lipid ratio threshold needed to deform (and eventually fuse) the bilayer.

Observing pore formation in the membrane requires proper experimental techniques monitoring this phenomenon. Apart from the early spectroscopic approaches, mainly based on monitoring the fluorescence of effluxed dyes in bulk experiments, there is a progress in applying the microscopic techniques allowing for single lipid vesicle analysis. In particular, a method called dual color single burst analysis (DCFBA), implemented on a confocal microscope setup, allowed for noticing a different

mode of action of a bee venom peptide- melittin, depending on a lipid composition (van den Bogaart et al., 2008) . It turned out that for liposomes composed of the zwitterionic DOPC, an increasing melittin to lipid ratio is needed for leakage of larger size-marker molecules, whereas for liposomes composed of the negatively charged DOPG, leakage is a-specific. The application of confocal imaging of single giant unilamellar vesicles allowed for comparing of all-or-none and graded permeabilization mechanisms induced by peptides derived from HIV fusion glycoprotein (Apellaniz et al., 2010). It seems that a properly applied and tailored experimental system may result in a precise characterization of peptide-induced pore formation.

We are aiming at:

- Creating coarse-grained (for membrane applications see Bond et al., 2007) and all-atom molecular models allowing for suitable and experimentally-relevant description of peptide-induced membrane phenomena

- Providing a quantitative experimental data on pore formation induced by peptides, especially by various forms of influenza virus fusion peptides

A PhD candidate will have an opportunity to work on an interdisciplinary project and will be performing experimental and computational work, balanced according to his/her previous experience. We believe that combining the experimental and computational approach can lead to better characterization of the subject. The computational part will be carried out at the Institute of Theoretical Physics, University of Warsaw under the supervision of dr hab. Piotr Szymczak and at the Institute of Physics, Polish Academy of Sciences under the supervision of prof. Marek Cieplak. The experimental part will be carried out under the supervision of dr Remigiusz Worch – a member of prof. Cieplak group.

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