The simulation studies of the interplay of peptides with lipids

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Chapter 5

Summary and Outlook

This thesis addresses the interplay between two classes of membrane active peptides and membranes, namely the antimicrobial peptides Magainin 2, BP100, MSI-78, and MSI-103 and the amphipathic peptide Pex11-Amph, by using molecular dynamics (MD) simulations. Understanding the interplay of peptides with membrane lipids is important to provide fundamental insight into many biological processes.

Antimicrobial peptides (AMPs), which are typically short and cationic peptides, are involved in antimicrobial defense mechanisms. Their broad spectrum of antimicrobial activity and potential role as a promising substitute for conventional antibiotics has attracted many people to study the interaction of AMPs with membranes both theoretically and experimentally. In **Chapter 2**, we presented a multiscale MD study of the lipid binding and segregation mechanism of AMPs, by employing the AMPs Magainin 2, BP100, and MSI-103 in interaction with POPE/cardiolipin mixed bilayers. In agreement with experimental observations, the more cationic peptides caused a higher segregation of anionic lipids from zwitterionic ones. The detail afforded by MD simulations shows that this action is indeed mediated mainly by electrostatic interactions whereby the first lipid shell around the peptides becomes enriched in cardiolipin. There is no indication that AMP-mediated anionic lipid segregation extended further than this annular shell, i.e., no higher order organization into cardiolipin domains was observed. Consistent with experimental observations, BP100 falls outside the charge–segregation relationship. Contacts between cardiolipins and charged BP100 side chains did follow the same charge trend as for Magainin 2 and MSI-103. From these observations we assign the outlying behavior of BP100 to its higher-than-average cationic density, which prevents maximal contact with cardiolipins.

Understanding how lipid phase separated membranes might affect the behavior of AMPs is an important step to provide a better understanding of the action of AMPs in vivo. In **Chapter 3** we performed coarse grained (CG) simulations of AMPs on a phase-separated membrane to study their preference for different domains. AMPs Magainin 2, BP100, MSI-103 have been used, to give a diversity of peptides in sizes, charges and sequences in the simulations; MSI-78 serves as a control which has been studied in experiment. Two peptide concentrations were tested, at peptide-to-lipid ratios of 1/200 and 1/20. All the peptides displayed a clear preference for the liquid disordered (Ld) phase over the liquid ordered (Lo) one and for all peptides but Magainin 2 there was a further preference for the domain interface over the disordered phase bulk. In case of Magainin 2, which occurs as dimers, peptide–peptide association is more energetically favorable in the Ld phase, somewhat less in the interface region, and energetically neutral in the Lo region, explaining the

preference of Magainin 2 for the Ld phase over the domain interface. Interestingly at high P/L ratio, two Magainin 2 pores spontaneously formed in the Ld phase. From the peptide's mechanistic point of view, this is an expected event. However, it is a remarkable observation because simulation of spontaneous AMP membrane insertion and pore formation has remained elusive for coarse-grain models, and descriptions of the process are only available from atomistic simulations. Even though it is reported that CG membranes based on the MARTINI force field often present an excessively high energy barrier to lipid flip-flop or crossing by polar moieties, it is not clear yet why MARTINI membranes are reluctant to form pores. In the MARTINI model, Magainin 2 intends to make membrane buckle and bud instead of pore formation. In our simulations at high peptide densities membrane buckling was prevented by the application of a restraining potential in the *z* direction on the lipid headgroups. One could argue that the pores were caused by the artificial restraint. To test the influence of this bias we employed a different method, namely a flat-bottomed potential, to restrict membrane buckling. Eventually we still observed two pores in the membrane. In the future, it would be very interesting to do more tests concerning the pore phenomena. Since all these pores formed in the Ld phase, which is mainly composed of DLiPC lipids, what would happen if we put Magainin 2 on a pure DLiPC bilayer? Do cholesterol or DPPC lipids play a role, e.g. by causing membrane area defects? What is the absolute threshold concentration of Magainin 2 to cause pore formation in the Ld phase?

Pex11-Amph is the amphipathic peptide involved in membrane remodelling during peroxisomal fission. As in case of most peptides, the mechanism of Pex11-Amph peptides inducing curvature in membranes is not clear yet. We performed simulations of *P. chrysogenum* Pex11-Amph peptide on different types of membranes using molecular dynamics in **Chapter 4**. Interestingly we observed peptides aggregating in a linear pattern on the membrane. One mutant of the peptide was successfully designed to break this aggregation pattern; the mutant's in vitro tubulating activity was also abolished, pointing towards a link between the two phenomena. By combining experiment and simulations we are able to shed light on the action of Pex11-Amph on the peroxisomal membrane. Apart from current experimental data, it would be interesting to get more clear measurements about the different behavior of the Pex11-Amph and arginine mutant, for instance, use electron microscopy to check the liposomes' shape after the binding of wide type peptide and arginine mutant, and see their corresponding behaviors in *vivo*.

Above all, I would say MD simulation is a very useful and an important tool to explore the interplay of peptides with membrane, providing a level of detail that cannot easily, or not at all, be obtained by experimental means. However, in the end, simulations and experiments working together is the most powerful approach to unravel the fascinating peptide-membrane interplay.